PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, C07K 14/50, A61K 38/18

A2

(11) International Publication Number: WO 99/55861

(43) International Publication Date: 4 November 1999 (04.11.99)

(21) International Application Number: PCT/JP99/02013

(22) International Filing Date: 15 April 1999 (15.04.99)

(30) Priority Data:
09/067,929 28 April 1998 (28.04.98) US

(71) Applicant: EISAI CO., LTD. [JP/JP]; 4-6-10, Koishikawa, Bunkyo-ku, Tokyo 112-8088 (JP).

(72) Inventors: ZHU, Hengyi; 4941 Brookburn Drive, San Diego, CA 92130 (US). KALYANARAMAN, Ramnarayan; 11674 Springside Road, San Diego, CA 92128 (US).

(74) Agents: KAWAGUCHI, Yoshio et al.; Yamada Building, 1-14, Shinjuku 1-chome, Shinjuku-ku, Tokyo 160-0022 (JP).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: FIBROBLAST GROWTH FACTOR MUTEIN COMPOSITIONS AND METHODS OF USE THEREFOR

(57) Abstract

Isolated nucleic acid encoding FGF mutein polypeptides, the mutein polypeptides and compositions containing the mutein polypeptides are provided. FGF mutein polypeptides that exhibit increased binding affinity for FGF receptors and reduced mitogenic activity are provided, and may be used in methods for treating FGF-mediated disorders, such as ophthalmic disorders, tumorigenic disorders and restenosis. Also provided are FGF mutein polypeptides that exhibit reduced receptor binding activity, but retain the ability to bind to heparin. Methods for treating heparin-related disorders by administering a therapeutically effective amount of an FGF mutein are also provided.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

BNSDOCID <WO 9955861A2 I >

DESCRIPTION

FIBROBLAST GROWTH FACTOR MUTEIN COMPOSITIONS AND METHODS OF USE THEREFOR

RELATED APPLICATIONS

This application is related to U.S. application Serial No. 09/067,929, to Zhu et al., entitled "FIBROBLAST GROWTH FACTOR MUTEIN COMPOSITIONS AND METHODS OF USE THEREFOR", filed April 28, 1998. Priority is claimed herein to the above application, the disclosure of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to mutein fibroblast growth factor (FGF) polypeptides and nucleic acids encoding the mutein FGF polypeptides. In particular, DNA encoding mutein FGF polypeptides, the mutein FGF polypeptides and compositions containing the mutein FGF polypeptides are provided. The mutein FGF polypeptides can be used in methods of modulating the activity of members of the FGF family polypeptides and in methods of treating heparin-associated disorders.

BACKGROUND OF THE INVENTION

During the last thirty years, a great deal of attention has been directed towards the identification and characterization of factors that stimulate the growth, proliferation and differentiation of specific cell types. Numerous growth factors and families of growth factors that share structural and functional features have been identified. Many of these factors have multifunctional activities and affect a wide spectrum of cell types.

Fibroblast growth factors and fibroblast growth factor receptors

One family of growth factors that has a broad spectrum of activities is the fibroblast growth factor (FGF) family [e.g., see Johnson *et al.*, Advan. Cancer Res. 60:1-41 (1993)] This family of proteins includes FGFs designated FGF-1 through FGF-12 (or acidic FGF (aFGF),

basic FGF (bFGF), int-2, hst/K-FGF, FGF-5, FGF-6, keratinocyte growth factor (KGF), FGF-8, FGF-9, FGF-10, FGF-11 and FGF-12, respectively). Acidic and basic FGF, which were the first members of the FGF family that were characterized, are about 55% identical at the amino acid level and are highly conserved among species. Basic FGF has a molecular weight of approximately 16 kD, is basic and temperature sensitive and has a high isoelectric point [pI = 9.6; e.g., see in The Cytokine *Facts*Book, Callard and Gearing, eds., p.121, Academic Press, Inc., London]. Acidic FGF has an acidic isoelectric point with a pI of about 5.4. The other members of the FGF family have subsequently been identified on the basis of amino acid sequence homologies with aFGF and bFGF and common physical and biological properties. These proteins are widely distributed in tissues, such as the central and peripheral nervous system, retina, kidney and myocardium.

In addition, FGFs have extremely high affinities for heparin, which is a highly sulfated, negatively charged polysaccharide, and many of the key amino acid residues required for heparin binding have been identified (Presta (1992) Biochem. Biophys. Res. Commun. 185:1098-1107; Thompson *et al.* (1994) Biochemistry 33:3831-3840; Li *et al.* (1994) Biochemistry 33:10999-11007). For example, aFGF and bFGF possess two potential binding domains for heparin, one located near the amino-terminal region, and the other near the carboxy-terminal region (residues 18-22 and 107 to 110 for bFGF and 9-12 and 100-102 for aFGF; e.g., see Gospodarowicz *et al.* (1987) Endocrin. Rev. 8:95-114; Baird *et al.* (1988) Proc. Natl. Acad. Sci. U.S.A. 85:2324-2328).

Although heparin binding is not absolutely required for the binding of an FGF to its receptor, heparin has been reported to modulate one or more activity of FGFs including increasing receptor affinity, conferring protection from heat and acid inactivation and proteolytic degradation,

BNSDOCID <WO 9955861A2 >

and is also essential for the mitogenic activity of bFGF stimulated cells (e.g., see Shi *et al.* (1993) Mol. Cell. Biol. 13:3907-3918; Roghani *et al.* (1994) J. Biol. Chem. 269:3976-3984; Gospodarowicz *et al.* (1986) J. Cell Biol. 128:475-484; Yanyon *et al.* (1991) Cell 64:841-848).

FGFs exhibit a mitogenic effect on a wide variety of mesenchymal, endocrine and neural cells. They are also important in differentiation and development. Of particular interest is their stimulatory effect on collateral vascularization and angiogenesis. Such effects have stimulated considerable interest in FGFs as therapeutic agents, for example, as pharmaceuticals for wound healing, neovascularization, nerve regeneration and cartilage repair.

The effects of FGFs are mediated by high affinity receptor tyrosine kinases on the cell surface membranes or FGF-responsive cells [e.g., see Lee *et al.*, (1989) Science 245, 57-60; Imamura *et al.*, B.B.R.C. 155, 583-590 (1989); Huang and Huang, (1986) J. Biol. Chem. 261, 9568-9571; Moscatelli, (1987) J. Cell. Physiol. 131, 123-130; Verdier *et al.* (1997) Genomics 40, 151-154; U.S. Patent No. 5,288,855]. Lower affinity receptors also play a role in mediating FGF activities. The high affinity receptor proteins constitute a family of structurally related FGF receptors (FGFRs). Four FGF receptor genes have been identified and at least two of these genes generate multiple mRNA transcripts via alternative splicing of the primary transcript [e.g., see U.S. Patent No. 5,288,855; Kiefer et al., (1991) Growth Factors 5:115-127]. This splicing potentially creates a large number of different molecular forms that can interact with FGF family members, thereby permitting cells to respond to different FGF family members. For example, alternative splicing of a single gene results in the receptor FGFR2, which has high affinity for acidic and basic FGFs but no detectable affinity for KGF, and the KGF receptor, which has high affinity for KGF but reduced affinity for

basic FGF. Similarly, alternative splicing of FGFR1 produces variants that have about a 50-fold decreased the affinity for basic FGF, but unchanged acidic FGF binding.

Receptor expression is altered by physical, chemical, and hormonal injury as well as in certain pathological conditions such as restenosis, tumors and selected proliferative diseases. Receptor messenger RNA and protein is expressed in melanoma cells (see, e.g., Becker et al. (1992) Oncogene 7: 2303-2313). The receptor message is not normally expressed in the palmar fascia, but is present in the proliferative hand disease Dupuytren's Contracture, (see, e.g., Gonzales et al. (1992) Amer. J. Pathol. 141: 61-671). Quiescent smooth muscle cells (SMCs) do not respond to bFGF, but proliferating SMCs, in a model of restenosis after balloon angioplasty, strongly respond to exogenous bFGF (see, e.g., Casscells et al. (1992) Proc. Natl. Acad. Sci. U.S.A. 89:7159-7163).

In addition to potentially useful proliferative effects, FGF-induced mitogenic stimulation may, in some instances, be detrimental. For example, cell proliferation and angiogenesis are an integral aspect of tumor growth. Members of the FGF family, including bFGF, are thought to play a pathophysiological role, for example, in tumor development, rheumatoid arthritis, proliferative diabetic retinopathies and other complications of diabetes. Because FGFs are associated with many disease states, they are therapeutic targets. For example, antagonists of bFGF activity and/or aFGF or other FGFs should have a therapeutic use in treatment of tumorigenic conditions, restenosis, and other such conditions in which an FGF polypeptide plays a pathogenic role.

BNSDOCID <WO 9955861A2 I >

Thus, there is interest in developing FGF-specific pharmacological products that modulate the activity of one or more FGF polypeptides.

Heparin-induced thrombosis and thrombocytopenia

Coronary artery thrombosis plays a pivotal role in the pathogenesis of acute coronary syndromes including, but not limited to: unstable angina, non Q-wave myocardial infarction and sudden death. Thrombotic occlusion of the artery is thought to be responsible for most of the acute manifestations of coronary artery diseases. As a result, antithrombotic therapy is a mainstay in the early management and treatment of patients suffering from acute coronary syndromes (*e.g.*, see van den Bos *et al.* (1993) Circulation 88:2058-2066; Bombardini *et al.* (1997) Angiology 48:969-976; Walenga *et al.* (1997) Curr. Opin. Pulm. Med. 3:291-302).

Heparin is the most widely used antithrombotic agent for acute management of thrombosis and is the treatment of choice for preventing and treating venous thromboembolism. The anticoagulant effect of heparin is not linked to a cellular target but is presumed to be exerted in conjunction with antithrombin III to inhibit the activity of soluble circulatory enzymes involved in the blood clotting cascade, particularly Factor Xa and Factor IIa.

Although heparin is widely used as the injectable anticoagulant of choice, it has several potential short comings. For example, the systemic administration of high levels of heparin used to impede local thrombus deposition also can results in the global reduction in Factor Xa and/or Factor IIa activity. A complication of systemic heparin therapy is severe bleeding in patients because of the reduced capability of blood to coagulate (e.g., Visentin *et al.* (1995) Curr. Opin. Hematol. 2:351-357). Severe bleeding is a serious thromboembolic complication of heparin

therapy and can result in crippling disabilities and/or death (e.g., see Sodian *et al.* (1997) ASAIO J. 43:M430-M433).

A notorious complication of systemic heparin therapy is heparin-induced thrombocytopenia. Heparin-induced thrombocytopenia (HIT) is an immunoglobulin-mediated adverse drug reaction associated with a high risk of thrombotic complications. The pathogenic antibody, usually immunoglobulin (Ig)G (HIT-IgG), recognizes a multimolecular complex of heparin and platelet factor 4, a heparin-binding protein normally contained in platelet alpha granules, resulting in platelet activation via platelet Fc receptors. There are an array of disorders or side-effects of heparin treatment that require treatment.

Thus, there is a need to develop pharmacological products that modulate the activity of FGFs and of heparin. Therefore it is an object herein to provide FGF polypeptide muteins and compositions containing these FGF muteins that modulate the activity of endogenous FGF polypeptides. It also an object herein to provide methods for modulating the activity of FGF polypeptides. It is also an object herein to provide methods for ameliorating FGF-mediated or related conditions, such as restenosis, tumorigenesis and other conditions involving angiogenesis and undesirable proliferation of fibroblasts.

It is also an object herein to provide mutagenized FGF peptides and compositions containing these FGF mutein peptides that modulate, particularly inhibit, the activity of heparin. It also an object herein to provide methods for ameliorating heparin-induced or heparin-related conditions, such as modulators of heparin-associated bleeding, antagonists of heparin-induced angiogenesis in ophthalmic disorders, and for treating heparin-induced thrombocytopenia and thrombosis.

BNSDOCID <WO 9955861A2 I >

SUMMARY OF THE INVENTION

Isolated nucleic acid encoding mutein FGF polypeptides, the mutein FGF polypeptides and compositions containing the mutein FGF polypeptides are provided. The mutein FGF polypeptides are useful in methods of modulating the activity of a FGF polypeptide, methods of modulating the activity of heparin and can be used for treating FGF-mediated or heparin-related disorders.

Nucleic acid encoding FGF mutein polypeptides that exhibit decreased mitogenic activity compared to wild type, but comparable or increased receptor binding affinity for one or more FGF receptors are provided. Such FGF polypeptides should be useful as competitive inhibitors of FGF activities. In particular, the mitogenic activity is reduced at least 100%, preferably at least about 2-fold, more preferably at least about 5-fold or even more preferably 5- to 15-fold, compared to the corresponding wild-type. Because of the reduced mitogenic activity, the resulting muteins can be used as competitive inhibitors of the wild-type, native or endogenous FGF polypeptides. Increasing the binding activity renders the resulting mutein more advantageous for use as a competitive inhibitor.

In preferred embodiments, the nucleic acid encodes a mutein of any of FGF-1 through FGF-12. The nucleic acid sequence of FGF-1 through FGF-12 is set forth in SEQ ID NOs. 1-10, respectively. The mutein polypeptides contain an amino acid replacement corresponding (by alignment of conserved amino acid residues) to position 138 of FGF-2. In one embodiment, nucleic acid molecules encoding FGF muteins having amino acid substitutions, preferably alanine or a conservative amino acid substitution therefor, corresponding to position Leu138 of FGF-2 (basic FGF) are provided.

Presently preferred nucleic acid molecules encode FGF-2 (bFGF) muteins that contain a glycine, serine or alanine residue at amino acid position 138 such that the resulting mutein has reduced mitogenic activity, and appear to have increased receptor binding activity compared to wild-type.

Also provided is nucleic acid encoding FGF muteins that bind to heparin but have little or substantially reduced FGF receptor binding activity compared to wild type. In particular, DNA encoding FGF muteins having amino acid substitutions, preferably alanine or a conservative amino acid substitution therefor, corresponding to positions 88 and 93 of FGF-2 (basic FGF) are provided. In addition, the muteins will optionally include replacement of the glu96 residue. In preferred embodiments, the DNA encodes an FGF-1 through FGF-10 set forth in SEQ ID NOs. 1-10, respectively, that contains an amino acid replacement corresponding (by alignment of conserved residues) to position 88 and 93 of FGF-2 or combinations thereof and optionally additionally replacement of Glu96, which is highly conserved among FGF peptides. Presently preferred FGF muteins are FGF-2 (bFGF) muteins in which the replacement amino acid is glycine, serine, alanine, methionine, leucine or tyrosine such that the resulting mutein retains heparin binding ability but has reduced, substantially reduced, preferably at least about 10-fold, more preferably at least about 100-fold or more, binding affinity for FGF receptors, particularly FGFR1 (for FGF-2) compared to the corresponding wild type FGF.

In other embodiments, the above-described nucleic acid encodes FGF muteins that further include replacement, preferably with serine, of one or more cysteine residues selected to increase stability, decrease aggregation increase solubility and increase homogeneity of recombinantly produced proteins by reducing or eliminating disulfide

BNSDOCID <WO 9955861A2 I >

scrambling. For example, this is achieved by replacing Cys69 and Cys87 in FGF-2 with serine. Replacement is preferably with a neutral amino acid, such as serine.

Also contemplated are nucleic acids that encode FGF muteins in which glycosylation sites are added to improve stability of the resulting polypeptide (see, U.S. Patent No. 5,464,943). The nucleic acids encoding the above-described FGF muteins can be modified by the introduction of one or more sequences encoding a glycosylation site into the coding region of an FGF mutein at a site that does not alter the biological properties of the encoded FGF mutein.

The encoded FGF mutein polypetides and compositions containing the polypeptides are also provided. FGF mutein polypeptides with amino acid replacements corresponding to position 138 of FGF-2 are provided. Particularly preferred are FGF mutein polypeptides in which the residue corresponding to position 138 of bFGF is replaced with glycine, serine or alanine, more preferably alanine. Such mutein FGF polypeptides have decreased mitogenic activity compared to wild type, but comparable or increased receptor binding affinity for one or more FGF receptors. When such muteins are formulated for pharmaceutical use, they can be used to prevent or inhibit the undesired growth and proliferation of FGF-responsive cells. Such cells occur in vascular disorders characterized by accelerated smooth muscle cell proliferation, such as rheumatoid arthritis, tumor angiogenesis, Kaposi's sarcoma, restenosis, In-stent restenosis, certain ophthalmic disorders and dermatological disorders, such as psoriasis.

FGF mutein polypeptides with amino acid replaced at positions that correspond one or more of positions 88 and 93 and optionally 96 of FGF-2 are provided. When such polypeptides are formulated for pharmaceutical use, they can be used as coagulants for heparin-

associated bleeding, antagonists of heparin-induced angiogenesis, and for treating heparin-induced thrombocytopenia and thrombosis. Particularly preferred are FGF-2 mutein peptides in which the Glu96 and Phe93 are replaced with glycine, serine or alanine, more preferably alanine.

Pharmaceutical compositions containing a therapeutically effective amount of one of these FGF muteins are provided. These composition can be used treating FGF-related disorders and heparin-related disorders. The compositions may be formulated for oral, intravenous or parenteral administration or in any suitable vehicle. The pharmaceutical compositions can be formulated for in a vehicle suitable for topical, local or systemic administration depending of the intended use. For example, the compositions may be formulated for administration sublingually, as aerosols, as suppositories, and for ophthalmic application.

Methods of modulating the activity of an FGF polypeptide are provided. Methods of treating of FGF-mediated disorders are also provided. In particular, methods of treating FGF-mediated disorders such as FGF-mediated restenosis, vascular injury, rheumatoid arthritis and FGF-mediated tumor angiogenesis by inhibiting binding of an FGF polypeptide to an FGF receptor or by antagonizing the mitogenic activity of an FGF are provided. In preferred methods, the methods use an effective amount of an FGF mutein polypeptide in which the residue corresponding to position 138 is replaced with glycine, serine phenylalanine, methionine, tyrosine or alanine, more preferably alanine.

Methods of treating heparin-related disorders by administering a therapeutically effective amount of an FGF mutein that binds to heparin but does not bind to its cognate receptor are also provided. In particular, methods of treating heparin-related disorders such as excessive bleeding resulting from the anticoagulant activity of the systemic administration of

BNSDOCID <WO 9955861A2 I >

heparin, heparin-induced and heparin-associated thrombocytopenia and thrombosis or the undesired stimulation of angiogenesis mediated by the interaction of heparin with an FGF, such as, for example, FGF-2, are provided.

Articles of manufacture containing packaging material, an FGF mutein polypeptide provided herein, which is effective for ameliorating the symptoms of a FGF-mediated or heparin-related disorder, antagonizing the effects of one or more FGF or inhibiting binding of an FGF polypeptide to an FGF receptor, antagonizing the effects of heparin or heparin binding to an endogenous FGF polypeptide, within the packaging material, and a label that indicates that the FGF mutein is used for antagonizing the effects of an FGF polypeptide or heparin, treating a FGF-mediated or heparin-related disorder, inhibiting the binding of a FGF polypeptide to an FGF receptor, or inhibiting the binding of heparin to an FGF peptide are provided.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. All patents and publications referred to herein are incorporated by reference herein.

The amino acids, which occur in the various amino acid sequences appearing herein, are identified according to their well-known, three-letter or one-letter abbreviations. The nucleotides, which occur in the various DNA fragments, are designated with the standard single-letter designations used routinely in the art.

As used herein, FGF refers to polypeptides that have amino acid sequences of native FGF proteins or conservative variants and allelic variants thereof. Such polypeptides include, but are not limited to, FGF-

1 - FGF-12. For example, bFGF (FGF-2) should be generally understood to refer to polypeptides having substantially the same amino acid sequence and receptor-targeting activity as that of bovine bFGF or human bFGF. It is understood that differences in amino acid sequences can occur among FGFs of different species as well as among FGFs from individual organisms or species. Reference to FGFs is also intended to encompass proteins isolated from natural sources as well as those made synthetically, as by recombinant means or possibly by chemical synthesis.

As used herein, an FGF mutein is a polypeptide member of the FGF family of peptides that contains at least one amino acid residue that differs from wild type or naturally-occurring FGF polypeptides. Among the preferred mutein polypeptides provided herein, are the FGF muteins that have replacements in amino acid residues at position 138 of bFGF. Preferred replacement amino acids are alanine, phenylalanine, serine, glycine and methionine, more preferably alanine, serine and glycine.

For purposes herein, reference is made the positions in FGF-2. Corresponding positions in other FGF polypeptides may be determined by sequence comparison in which homologous regions are aligned. With respect to the FGF family, such alignment is well known to those of skill in the art. Identification of corresponding residues is exemplified herein. It is noted that requiring replacement of an amino acid means that the amino acid present in the wild type or native polypeptide is replaced with a different amino acid from the naturally occurring amino acid.

Other positions may also be replaced with conservative amino acid substitutions that do not substantially alter activity. Suitable conservative substitutions of amino acids are known to those of skill in this art and may be made generally without altering the biological activity of the resulting molecule. Those of skill in this art recognize that, in

BNSDOCID <WO 9955861A2 1 >

general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g., Watson et al. *Molecular Biology of the Gene*, 4th Edition, 1987, The Bejacmin/Cummings Pub. co., p.224).

Such substitutions are preferably made in accordance with those set forth in TABLE 1 as follows:

TABLE 1

Original residue	Conservative substitution
Ala (A)	Gly; Ser
Arg (R)	Lys
Asn (N)	Gln; His
Cys (C)	Ser
Gln (Q)	Asn
Glu (E)	Asp
Gly (G)	Ala; Pro
His (H)	Asn; Gln
Ile (I)	Leu; Val
Leu (L)	Ile; Val
Lys (K)	Arg; Gln; Glu
Met (M)	Leu; Tyr; Ile
Phe (F)	Met; Leu; Tyr
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr
Tyr (Y)	Trp; Phe
Val (V)	Ile; Leu

Other substitutions are also permissible and may be determined empirically or in accord with known conservative substitutions.

As used herein, nucleic acid encoding an FGF polypeptide or polypeptide reactive with an FGF receptor refers to any of the nucleic acid molecules set forth herein as coding such peptides, to any such nucleic acid molecules known to those of skill in the art, any nucleic acid that encodes an FGF and any nucleic acid encoding an FGF that can be isolated from a human cell library using any of the preceding nucleic acid molecules or fragments thereof as a probe. Native FGFs are encoded by any nucleic acid that encodes any of the FGF polypeptides set forth in

SEQ ID NOs. 1-10 (such nucleic acid sequences are available in publicly accessible databases, such as DNA* (July, 1993 release from DNASTAR, Inc. Madison, WI; see, also U.S. Patent No. 4,956,455, U.S. Patent No. 5,126,323, U.S. Patent No. 5,155,217, U.S. Patent No. 4,868,113, published International Application WO 90/08771, which is based on U.S. Application Serial No. 07/304,281, filed January 31, 1989, U.S. Patent No. 5,731,170, U.S. Patent No. 5, 707,805, U.S. application Serial No. 5,665,870 and Miyamoto et al. (1993) Mol. Cell. Biol. 13:4251-4259), and any DNA fragment that may be produced from any of the preceding DNA fragments by substitution of degenerate codons. It is understood that once the complete amino acid sequence of a peptide, such as an FGF polypeptide, and one nucleic acid molecule encoding such peptide are available to those of skill in this art, it is routine to substitute degenerate codons and produce any of the possible molecules that encode such peptide. It is also generally possible to synthesize nucleic acid molecules encoding such peptide based on the amino acid sequence.

As used herein, vector or plasmid refers to discrete elements that are used to introduce heterologous DNA into cells for either expression of the heterologous DNA or for replication of the cloned heterologous DNA. Selection and use of such vectors and plasmids are well within the level of skill of the art.

As used herein, expression vector includes vectors capable of expressing DNA fragments that are in operative linkage with regulatory sequences, such as promoter regions, that are capable of effecting expression of such DNA fragments. Thus, an expression vector refers to a recombinant DNA or RNA construct, such as a plasmid, a phage, recombinant virus or other vector that, upon introduction into an appropriate host cell, results in expression of the cloned DNA.

BNSDOCID <WO 9955861A2 I >

Appropriate expression vectors are well known to those of skill in the art and include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or may integrate into the host cell genome.

As used herein, operative linkage or operative association of heterologous DNA to regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences, refers to the functional relationship between such DNA and such sequences of nucleotides. For example, operative linkage of heterologous DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA in reading frame.

As used herein, a promoter region refers to the portion of DNA of a gene that controls transcription of DNA to which it is operatively linked. A portion of the promoter region includes specific sequences of DNA that are sufficient for RNA polymerase recognition, binding and transcription initiation. This portion of the promoter region is referred to as the promoter. In addition, the promoter region includes sequences that modulate this recognition, binding and transcription initiation activity of the RNA polymerase. These sequences may be cis acting or may be responsive to trans acting factors. Promoters, depending upon the nature of the regulation, may be constitutive or regulated. For use herein, inducible promoters are preferred. The promoters are recognized by an RNA polymerase that is expressed by the host. The RNA polymerase may be endogenous to the host or may be introduced by genetic engineering into the host, either as part of the host chromosome or on an episomal element.

As used herein, transfection refers to the taking up of DNA or RNA by a host cell. Transformation refers to this process performed in a manner such that the DNA is replicable, either as an extrachromosomal element or as part of the chromosomal DNA of the host. Methods and means for effecting transfection and transformation are well known to those of skill in this art (see, e.g., Wigler et al. (1979) Proc. Natl. Acad. Sci. USA 76:1373-1376; Cohen et al. (1972) Proc. Natl. Acad. Sci. USA 69:2110).

As used herein, heparin is the heterogenous, sulfated anionic polysaccharide composed of D-iduronate-2-sulfate and *N*-sulfo-D-glucosamine-6-sulfate bound to a protein core as the "proteoglycan" or in a free form that has potent anti-coagulant activity.

As used herein, heparin-like substances are molecules that have oligosaccharide structures related to heparin and exhibit an anti-coagulant activity of substantially similar to heparin.

As used herein, a heparin-induced or heparin-related disorder is a disorder in which the administration of heparin or heparin-like substances causes or contributes to the pathology or adverse effects thereof. Such disorders include, but are not limited to: proliferative disorders arising from heparin-induced, FGF-mediated angiogenesis, heparin-induced and heparin-associated thrombocytopenia and thrombosis and excessive bleeding caused by or associated with the anti-coagulant activity of heparin.

As used herein, an FGF-mediated disorder is a disorder in which FGF causes or contributes to the pathology. Such disorders include, but are not limited to: restenosis, diabetic retinopathies, tumorigenesis, ophthalmic disorders and other proliferative disorders.

As used herein, treatment means any manner in which the symptoms or pathology of a condition, disorder or disease are

BNSDOCID <WO 9955861A2 I >

ameliorated or otherwise beneficially altered. Treatment also encompasses any pharmaceutical use of the compositions herein.

As used herein, amelioration of the symptoms of a particular disorder by administration of a particular pharmaceutical composition refers to any lessening, whether permanent or temporary, lasting or transient that can be attributed to or associated with administration of the composition.

As used herein, local application or administration refers to administration of an anti-hyperalgesic agent to the site, such as an inflamed joint, that exhibits the hyperalgesic condition and that does not exert central analgesic effects or CNS effects associated with systemic administration of opioids that cross the blood brain barrier. Such local application includes intrajoint, such as intra-articular application, via injection, application via catheter or delivery as part of a biocompatible device.

As used herein, topical application refers to application to the surface of the body, such as to the skin, eyes, mucosa and lips, which can be in or on any part of the body, including but not limited to the epidermis, any other dermis, or any other body tissue. Topical administration or application means the direct contact of the anti-hyperalgesic with tissue, such as skin or membrane, particularly the cornea, or oral, vaginal or buccal mucosa. Topical administration also includes application to hardened tissue such as teeth and appendages of the skin such as nails and hair. A composition formulated for topical administration is generally liquid or semi-liquid carriers such a gel, lotion, emulsion, cream, plaster, or ointment, a spray or aerosol, or a "finite" carrier, i.e., a non-spreading substance that retains its form, such as a patch, bioadhesive, dressing and bandage. It may be aqueous or non-aqueous; it may be formulated as a solution, emulsion or a suspension.

As used herein, biological activity refers to the in vivo activities of a compound or physiological responses that result upon in vivo administration of a compound, composition or other mixture. Biological activity, thus, encompasses therapeutic effects and pharmaceutical activity of such compounds, compositions and mixtures. Biological activity may be detected by in vitro assays, such as those described herein.

As used herein, an effective amount of a compound for treating a disorder is an amount that is sufficient to ameliorate, or in some manner reduce a symptom or stop or reverse progression of a condition. Such amount may be administered as a single dosage or may be administered according to a regimen, whereby it is effective.

As used herein, pharmaceutically acceptable salts, esters or other derivatives of the compounds include any salts, esters or derivatives that may be readily prepared by those of skill in this art using known methods for such derivatization and that produce compounds that may be administered to animals or humans without substantial toxic effects and that either are pharmaceutically active or are prodrugs. For example, hydroxy groups can be esterified or etherified.

As used herein, substantially pure means sufficiently homogeneous to appear free of readily detectable impurities as determined by standard methods of analysis, such as thin layer chromatography [TLC], gel electrophoresis and high performance liquid chromatography [HPLC], used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not detectably alter the physical and chemical properties, such as enzymatic and biological activities, of the substance. Methods for purification of the compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure

BNSDOCID <WO 9955861A2 I >

compound may, however, be a mixture of stereoisomers. In such instances, further purification might increase the specific activity of the compound.

As used herein, adequately pure or "pure" per se means sufficiently pure for the intended use of the adequately pure compound.

As used herein, a prodrug is a compound that, upon in vivo administration, is metabolized or otherwise converted to the biologically, pharmaceutically or therapeutically active form of the compound. To produce a prodrug, the pharmaceutically active compound is modified such that the active compound will be regenerated by metabolic processes. The prodrug may be designed to alter the metabolic stability or the transport characteristics of a drug, to mask side effects or toxicity, to improve the flavor of a drug or to alter other characteristics or properties of a drug. By virtue of knowledge of pharmacodynamic processes and drug metabolism in vivo, once a pharmaceutically active compound is identified, those of skill in the pharmaceutical art generally can design prodrugs of the compound [see, e.g., Nogrady (1985) Medicinal Chemistry A Biochemical Approach, Oxford University Press, New York, pages 388-392].

As used herein, the IC_{50} refers to an amount, concentration or dosage of a particular compound that achieves a 50% inhibition of a maximal response.

As used herein, EC_{50} refers to a dosage, concentration or amount of a particular test compound that elicits a dose-dependent response at 50% of maximal expression of a particular response that is induced, provoked or potentiated by the particular test compound.

As used herein, an FGF antagonist is a compound, such as a drug or an antibody, that inhibits FGF-mediated physiological responses. The antagonist may act by interfering with the interaction of FGF with the

FGF-specific receptor or by interfering with the physiological response to or bioactivity of an FGF polypeptide, such as cell proliferation. The effectiveness of a potential antagonist can be assessed using methods known to those of skill in the art. For example, the properties of a potential antagonist may be assessed as a function of its ability to compete with radiolabelled bFGF to bind to one or more FGF receptor using a purified FGF receptor binding assay or a cell-based receptor assay.

As used herein, an heparin antagonist is a compound, such as an FGF mutein described herein, that inhibits heparin-induced physiological responses. The antagonist may act by interfering with the interaction of heparin by for example, binding to and sequestering free heparin present in blood. The effectiveness of a potential heparin antagonist can be assessed using methods known to those of skill in the art. For example, the properties of a potential FGF mutein antagonist may be assessed as a function of its ability to bind to heparin and reduced ability to bind one or more FGF receptor using a purified FGF receptor binding assay or a heparin binding assay.

As used herein, replacement of an amino acid residue with another amino acid refers to the substitution of the amino acid residue at the specified position with an amino acid selected such that the resulting protein differs from the wild type protein.

As used herein, the abbreviations for any group or other compounds, are, unless indicated otherwise, in accord with their common usage, recognized abbreviations, or the IUPAC-IUB Commission on Biochemical Nomenclature (see, (1972) Biochem. 11:942-944).

A. Preparation of nucleic acid encoding FGF muteins

1. Nucleic acid encoding FGF polypeptides

DNA encoding an FGF polypeptide for mutagenesis reactions may be isolated, synthesized or obtained from commercial sources (the amino acid sequences of FGF-1 to FGF-10 are set forth in SEQ ID NOs. 1-10; DNA sequences may be based on these amino acid sequences or may be those that are known to those of skill in this art (see, e.g., DNA* (July, 1993 release from DNASTAR, Inc. Madison, WI); see, also U.S. Patent No. 4,956,455, U.S. Patent No. 5,126,323, U.S. Patent No. 5,155,217, U.S. Patent No. 4,868,113, U.S. Patent No. 5,514,516, published International Applications WO 95/24414 and WO 90/08771, U.S. Patent No. 5,731,170, U.S. Patent No. 5, 707,805, U.S. Patent No. 5,665,870 and Miyamoto et al. (1993) Mol. Cell. Biol. 13:4251-4259).

Specific reference to amino acid sequence positions of bFGF is relative to the 146 amino acid isoform of bFGF, which is generated from N-terminal truncation of the 155 amino acid isoform set forth in SEQ ID NO: 2 [e.g., see International application Publication No. WO 86/07595].

2. DNA constructs for recombinant production of FGF muteins

DNA is introduced into a plasmid for expression in a desired host. In preferred embodiments, the host is a bacterial host. The sequences of nucleotides in the plasmids that are regulatory regions, such as promoters and operators, are operationally associated with one another for transcription of the sequence of nucleotides that encode an FGF mutein. The sequence of nucleotides encoding the FGF mutein may also include DNA encoding a secretion signal, whereby the resulting peptide is a precursor of the FGF mutein.

In preferred embodiments the DNA plasmids also include a transcription terminator sequence. The promoter regions and

transcription terminators are each independently selected from the same or different genes.

A wide variety of multipurpose expression vectors suitable for the site-directed mutagenesis of heterologous proteins are known to those of skill in the art and are commercially available. Expression vectors containing inducible promoters or constitutive promoters that are linked to regulatory regions are preferred. Such promoters include, but are not limited to, the T7 phage promoter and other T7-like phage promoters, such as the T3, T5 and SP6 promoters, the trp, lpp, and lac promoters, such as the lacUV5, from E. coli; the P10 or polyhedron gene promoter of baculovirus/insect cell expression systems and inducible promoters from other eukaryotic expression systems.

Particularly preferred plasmids for transformation of E. coli cells include the pET expression vectors (see, U.S patent 4,952,496; available from NOVAGEN, Madison, WI). For example, the plasmid pET11d is a prokaryotic expression vector that contains a multiple cloning site for inserting heterologous DNA templates downstream of a bacteriophage T7 promoter. Transformation into a bacterial host that expresses T7 RNA polymerase, e.g., E. coli strain BL21(DE3), results in high level, recombinant expression of the heterologous protein.

As exemplified below, pET11d was used for the site-directed mutagenesis and intracellular expression of bFGF and bFGF muteins. For instance, a synthetic DNA encoding human bFGF [e.g., see SEQ ID NO:2; R & D Systems, Minneapolis, MN] was digested with the restriction endonucleases *Nco*I and *Bam*HI and placed in operable association with the T7 promoter by ligating into the *Nco*I and *Bam*HI of pET11d. The resulting plasmid was transformed in a competent bacteria host for recombinant expression of the encoded polypeptide.

DNA expression vectors encoding other FGF polypeptides [e.g.,

BNSDOCID <WO 9955861A2 I >

SEQ ID NOs:1 and 3-10] may be constructed using similar methods to those described herein or by using other methods and commercially available vectors known to those of skill in the art [see, e.g., Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY].

3. DNA mutagenesis

The introduction of a mutation into the coding region of an FGF polypeptide may be effected using any method known to those of skill in the art, including site-specific or site-directed mutagenesis of DNA encoding the protein. For example, site-directed mutagenesis may be performed as described herein or using mutagenesis kits available from a variety of commercial sources [e.g., see Clontech, Transformer Site-directed Mutagenesis Kit, Item No. PT1130-1].

Site-specific mutagenesis is typically effected using mesophilic or thermophilic PCR-based mutagenesis or using a phage vector that has single- and double-stranded forms, such as M13 phage vectors, which are well-known and commercially available. Other suitable phagemid vectors that contain a single-stranded phage origin of replication may be used (see, e.g., Veira et al. (1987) Meth. Enzymol. 15:3). In general, site-directed mutagenesis is performed by preparing a single-stranded vector that encodes the protein of interest (i.e., a member of the FGF family). An oligonucleotide primer that contains the desired mutation within a region of homology to the DNA in the single-stranded vector is annealed to the template followed by addition of a DNA polymerase, such as E. coli polymerase I Klenow fragment, which uses the double stranded region as a primer to produce a heteroduplex in which one strand encodes the altered sequence and the other the original sequence. The heteroduplex is introduced into appropriate bacterial cells and clones that include the desired mutation are selected. The encoded FGF mutein

may be expressed recombinantly in appropriate host organisms to produce the encoded polypeptide.

As exemplified below, site-directed mutagenesis was performed to introduce amino acid substitutions in residues corresponding to positions Tyr73, Val88 and Phe93 of bFGF. These residues were each changed to alanine residues following the site-directed mutagenesis procedure set forth below in Example 2A.2. The resulting muteins were then expressed and the Val88 and Phe93 were partially purified and can be purified to near homogeneity employing a heparin-Sepharose column followed by a CM-Sepharose column. The binding affinity of each of the above-described muteins to soluble FGFR1β-tissue plasminogen activator (tPA) fusion protein (see EXAMPLE 2A) was determined and compared with wild-type bFGF.

Site-directed mutagenesis was also performed to introduce amino acid substitutions in residues corresponding to positions phenylalanine 30 and leucine 138 of bFGF. Each of these residues was changed to an alanine following the site-directed mutagenesis procedure set forth below in Example 1A.2, the proteins were expressed, purified and the receptor binding activity was determined as in Example 2A.

B. Preparation of FGF mutein polypeptides

1. Host organisms for recombinant production of FGF muteins

Host organisms include those organisms in which recombinant production of heterologous proteins have been carried out, such as, but not limited to, bacteria (for example, E. coli), yeast (for example, Saccharomyces cerevisiae and Pichia pastoris), mammalian cells, insect cells. Presently preferred host organisms are strains of bacteria. Most preferred host organisms are strains of E. coli. Mammalian cells and insect cells are also contemplated for use in expressing the mutein FGF polypeptides provided herein.

Expression of a recombinant bFGF in yeast and E. coli is known to those of skill in the art (see, e.g., Barr *et al.* (1988) J. Biol. Chem. 263:16471-16478; and published International PCT Application Serial No. PCT/US93/05702). These methods may be adapted for expression of the mutein FGF polypeptides provided herein. Expression of DNA provided herein may also be performed as described herein. Available DNA encoding FGF polypeptides may be used as the starting materials for the producing the FGF polypeptides provided herein.

2. Methods for recombinant production of FGF muteins

The DNA encoding an FGF mutein is introduced into a plasmid in operative linkage to an appropriate promoter for expression of polypeptides in a selected host organism. The DNA fragment encoding the FGF mutein may also include a protein secretion signal that functions in the selected host to direct the mature polypeptide into the periplasm or culture medium. The resulting FGF mutein can be purified by methods routinely used in the art for wild type FGF, including, methods described hereinafter in the Examples.

Methods of transforming suitable host cells, preferably bacterial cells, and more preferably E. coli cells, as well as methods applicable for culturing the cells containing a gene encoding a heterologous protein, are generally known in the art. See, for example, Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Once the FGF mutein-encoding DNA fragment has been introduced into the host cell, the desired FGF mutein is produced by subjecting the host cell to conditions under which the promoter is induced, whereby the operatively linked DNA is transcribed. In a preferred embodiment, the promoter is the T7 RNA polymerase promoter and the E. coli host strain BL21(DE3) includes DNA encoding T7 RNA polymerase operably linked

to the lac operator and a promoter, preferably the lacUV5 promoter (see, e.g., Muller-Hill et al. (1968) Proc. Natl. Acad. Sci. USA 59:1259-12649). Addition of IPTG induces expression of the T7 RNA polymerase and the T7 promoter, which is recognized by the T7 RNA polymerase. In more preferred embodiments, the DNA construct includes a transcription terminator that is recognized by T7 RNA polymerase.

3. Preparation of FGF mutein polypeptides

Recombinantly expressed human FGF muteins may be purified according to standard methods used for the purification of the corresponding wild type FGFs [e.g., see Zhu *et al.* J. Biol. Chem. 270:21869-21871 (1995); U.S. Patent No. 5,120,715]. In addition, a variety of chromatographic methods, such as ion-exchange chromatography or immunoaffinity chromatography using antibodies raised against an FGF polypeptide, may also be used.

For exemplification, bFGF muteins in which amino acid residue Phe30, Tyr73, Val88, Phe93 and Leu138 have been replaced with alanine residues have been prepared following the methods and teachings described herein. The DNA encoding each of these human bFGF muteins was inserted in pET11d in operable association with the T7 promoter and the resulting plasmids were transformed into competent BL21(DE3). The expression of the FGF mutein was induced and the FGF muteins were and can be purified using ion-exchange chromatography. The bioactivity of each bFGF mutein was determined using one or more assay described herein. Other mutein polypeptides may be similarly prepared.

C. FGF muteins

Muteins of FGF family members, including FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9 and FGF-10 are provided. In particular, muteins include:

BNSDOCID <WO 9955861A2 I >

FGF-1 has been modified by replacement of the tyrosine residue at position 79 with another amino acid;

FGF-2 has been modified by replacement of the tyrosine residue at position 73 with another amino acid;

FGF-3 has been modified by replacement of the tyrosine residue at position 96 with another amino acid;

FGF-4 has been modified by replacement of the phenylalanine residue at position 135 with another amino acid;

FGF-5 has been modified by replacement of the phenylalanine residue at position 141 with another amino acid;

FGF-6 has been modified by replacement of the phenylalanine residue at position 128 with another amino acid;

FGF-7 has been modified by replacement of the tyrosine residue at position 118 with another amino acid;

FGF-8 has been modified by replacement of the tyrosine residue at position 107 with another amino acid;

FGF-9 has been modified by replacement of the tyrosine residue at position 115 with another amino acid; and

FGF-10 has been modified by replacement of the tyrosine residue at position 64 with another amino acid. The position numbers are determined by reference to SEQ ID NOS. 1 to 10 for FGF-1 to FGF-10, respectively.

Other muteins include:

FGF-1 has been modified by replacement of the proline residue at position 94 with another amino acid;

FGF-2 has been modified by replacement of the valine residue at position 88 with another amino acid;

FGF-3 has been modified by replacement of the tyrosine residue at position 111 with another amino acid;

FGF-4 has been modified by replacement of the phenylalanine residue at position 151 with another amino acid;

FGF-5 has been modified by replacement of the phenylalanine residue at position 156 with another amino acid;

FGF-6 has been modified by replacement of the phenylalanine residue at position 143 with another amino acid;

FGF-7 has been modified by replacement of the cysteine residue at position 133 with another amino acid;

FGF-8 has been modified by replacement of the lysine residue at position 123 with another amino acid;

FGF-9 has been modified by replacement of the leucine residue at position 130 with another amino acid;

FGF-10 has been modified by replacement of the phenylalanine residue at position 79 with another amino acid;

FGF-1 has been modified by replacement of the leucine residue at position 99 with another amino acid;

FGF-2 has been modified by replacement of the phenylalanine residue at position 93 with another amino acid;

FGF-3 has been modified by replacement of the glutamic acid residue at position 116 with another amino acid;

FGF-4 has been modified by replacement of the threonine residue at position 156 with another amino acid;

FGF-5 has been modified by replacement of the lysine residue at position 161 with another amino acid;

FGF-6 has been modified by replacement of the lysine residue at position 148 with another amino acid;

FGF-7 has been modified by replacement of the asparagine residue at position 138 with another amino acid;

BNSDOCID <WO 9955861A2 I >

FGF-8 has been modified by replacement of the valine residue at position 128 with another amino acid;

FGF-9 has been modified by replacement of the valine residue at position 135 with another amino acid; and

FGF-10 has been modified by replacement of the lysine residue at position 84 with another amino acid. The position numbers are determined by reference to SEQ ID NOS. 1 to 10 for FGF-1 to FGF-10, respectively.

Still further FGF muteins include:

FGF-1 has been modified by replacement of the phenylalanine residue at position 37 with another amino acid;

FGF-2 has been modified by replacement of the phenylalanine residue at position 30 with another amino acid;

FGF-3 has been modified by replacement of the lysine residue at position 53 with another amino acid;

FGF-4 has been modified by replacement of the phenylalanine residue at position 94 with another amino acid;

FGF-5 has been modified by replacement of the phenylalanine residue at position 99 with another amino acid;

FGF-6 has been modified by replacement of the phenylalanine residue at position 86 with another amino acid;

FGF-7 has been modified by replacement of the tryptophan residue at position 75 with another amino acid;

FGF-8 has been modified by replacement of the histidine residue at position 64 with another amino acid;

FGF-9 has been modified by replacement of the phenylalanine residue at position 72 with another amino acid;

FGF-10 has been modified by replacement of the tyrosine residue at position 21 with another amino acid;

FGF-1 has been modified by replacement of the leucine residue at position 146 with another amino acid;

FGF-2 has been modified by replacement of the leucine residue at position 138 with another amino acid;

FGF-3 has been modified by replacement of the leucine residue at position 177 with another amino acid;

FGF-4 has been modified by replacement of the histidine residue at position 201 with another amino acid;

FGF-5 has been modified by replacement of the histidine residue at position 214 with another amino acid;

FGF-6 has been modified by replacement of the histidine residue at position 193 with another amino acid;

FGF-7 has been modified by replacement of the histidine residue at position 187 with another amino acid;

FGF-8 has been modified by replacement of the lysine residue at position 176 with another amino acid;

FGF-9 has been modified by replacement of the histidine residue at position 186 with another amino acid; and

FGF-10 has been modified by replacement of the histidine residue at position 135 with another amino acid. The position numbers are determined by reference to SEQ ID NOS. 1 to 10 for FGF-1 to FGF-10, respectively.

In preferred embodiments, DNA encoding an FGF polypeptide is mutagenized to introduce an amino acid substitution at position corresponding to residue Leu138 of bFGF (FGF-2), such that the resulting peptide has decreased mitogenic activity compared to wild type, but comparable or increased receptor binding affinity for one or more FGF receptors. Preferably, the substituting amino acid residue is alanine or a conservative variant thereof.

BNSDOCID <WO 9955861A2 I >

In other preferred embodiments, DNA encoding an FGF polypeptide is mutagenized to introduce an amino acid substitution at positions corresponding to residues Tyr73, Val88 and Phe93 of bFGF (FGF-2), such that the resulting peptide has reduced binding to the cognate FGF receptor, but retains heparin binding activity. Preferably, the substituting amino acid residue is alanine or a conservative variant thereof. Muteins in which two or three of the above residues are modified are also provided herein. Also provided are muteins in which in addition to the above-noted modifications, also have the Glu at the position corresponding to the Glu96 in FGF-2 replaced, preferably with alanine, phenylalanine, serine or glycine.

Table 3 indicates the positions of the residues of FGF-1 through FGF-10 that correspond to the above-identified residues of bFGF as determined by the alignment of homologous regions of the sequence of amino acids set forth in SEQ ID NOs: 1-10.

TABLE 3

	Corresponding residue in bFGF					
FGF Member	Phe30	Tyr73	Val88	Phe93	Glu96	Leu138
1	Phe37	Tyr79	Pro94	Leu99	Glu102	Leu146
2	Phe30	Tyr73	Val88	Phe93	Glu96	Leu138
3	Lys53	Tyr96	Tyr111	Glu116	Glu119	Leu177
4	Phe94	Phe135	Phe151	Thr156	Glu159	His201
5	Phe99	Phe141	Phe156	Lys161	Glu164	His214
6	Phe86	Phe128	Phe143	Lys148	Glu151	His193

7	Trp75	Tyr118	Cys133	Asn138	Glu141	His187
8	His64	Tyr107	Lys123	Val128	Glu131	Lys176
9	Phe72	Tyr115	Leu130	Val135	Glu137	His186
10	Tyr21	Tyr64	Phe79	Lys84	Glu87	His135

In certain preferred embodiments, the FGF is FGF-2, is encoded by the DNA set forth in SEQ ID NO:2 and the replacement amino acid residue is glycine, serine or alanine. In more preferred embodiments, the substituting amino acid residue is alanine.

Also provided are muteins in which in addition to the above-noted modifications, also have one or more of the Cys69 and Cys87 of FGF-2 replaced with serine residues. For example, FGF-1 can be further modified by replacement of one or two of the cysteine residues at positions 31, 98 or 132; FGF-3 by replacement of the cysteine residue at position 50 or 115; FGF-4 by replacement of the cysteine residue at 88 or 155; FGF-5 has been by replacement of the cysteine residues at position 93, 160 or 202; FGF-6 by replacement of the cysteine at position 80 or 147; FGF-7 by replacement of the cysteine residues at position 18, 23, 32, 46, 71, 133 or 137; FGF-8 by replacement of the cysteine residues at position 10, 19, 109 or 127; FGF-9 by replacement of the cysteine residue at position 68 or 134; and FGF-10 by replacement of the cysteine residue at position 83 or 144.

In most preferred embodiments, the DNA encoding an FGF polypeptide encodes bFGF as set forth in SEQ ID NO:2 and amino acid residue leucine 138 is substituted with alanine, and optionally, depending upon intended use, with the replaced cysteine residues to decrease aggregation.

BNSDOCID <WO 9955861A2 I >

In other most preferred embodiments, the FGF has been mutagenized to introduce an amino acid substitution at positions corresponding Val88 or Phe93 and Glu96 of bFGF, such that the resulting peptide has reduced binding to the cognate FGF receptor, but retains heparin binding activity. Preferably, the substituting amino acid residue is alanine and optionally, depending upon intended use, with the replaced cysteine residues to decrease aggregation.

D. Evaluation of the bioactivity of FGF muteins

1. FGF receptor binding assays

Standard physiological, pharmacological and biochemical procedures are available for testing the FGF muteins to identify those that possess any biological activities that interfere with or inhibit FGF polypeptides. Numerous assays are known to those of skill in the art for evaluating the ability of FGF muteins to modulate the activity of one or more FGF polypeptide. For example, the properties of a potential antagonist may be assessed as a function of its ability to inhibit FGF activity including the ability in vitro to compete for binding to FGF receptors present on the surface of tissues or recombinant cell lines, cell-based competitive assays [see, e.g., Mostacelli *et al.* (1987) J. Cell. Physiol. 131:123-130]; mitogenic assays [Gospardarowicz *et al.* (1984) Proc. Natl. Acad. Sci. U.S.A. 81:6963-6967; Thomas *et al.* (1984) Proc. Natl. Acad. Sci. U.S.A. 81:357]; stimulation of angiogenesis in vitro [see, e.g., European Patent Application No. EP 645 451]; cell proliferation assays or heparin binding assays [see, e.g., International Application Publication No. WO 92/12245]; assays measuring the release of cellular proteases [Mostacelli *et al.* (1986) Proc. Natl. Acad. Sci. U.S.A. 83:2091-2095; Phadke (1987) Biochem. Biophys. Res. Comm. 142:448-453]; and, assays for the promotion of FGF-mediated neurite outgrowth and neuron survival [Togari *et al.* (1983) Biochem.

Biophys. Res. Comm. 114:1189-1193; Wagner *et al.* (1986) J. Cell Biol. 103:1363-1367].

In addition, FGF isotype specific antagonists may be identified by the ability of a sub-type specific FGF mutein to interfere with one or more FGF polypeptide binding to different tissues or cells expressing different FGF receptor subtypes, or to interfere with the biological effects of an FGF polypeptide [see, e.g., International Patent Application Publication No. WO 95/24414].

Using such assays, the relative affinities of the compounds for FGF receptors have been and can be assessed. Those that possess the desired in vitro properties, such as specific inhibition of the binding of bFGF, are selected. The selected FGF muteins that exhibit desirable activities, e.g., specifically inhibit binding of FGF to its receptor and has with substantially reduced biological activity, may be therapeutically useful in the methods described herein and are tested for such uses employing the above-described assays from which the in vivo effectiveness may be evaluated [Gospodarowicz *et al.* (1987) Endocrin. Rev. 8:95-114; Buntrock *et al.* (1982) Exp. Pathol. 21:62-67; International Patent Application Publication No WO 92/08473]. FGF muteins that exhibit the in vitro activities that correlate with the in vivo effectiveness will then be formulated in suitable pharmaceutical compositions and used as therapeutics.

2. Heparin binding assays

The heparin binding activity of the FGF muteins can be measured using the methods described herein or other methods known to those of skill in the art. For example, the ability of FGF muteins to bind to heparin can be determined by methods including, but not limited to, heparin or heparan sulfate or heparin Sepharose chromatography (Zhang *et al.* (1991) Proc. Natl. Acad. Sci. U.S.A. 88:3441-3445; International patent

BNSDOCID <WO 9955861A2 I >

application No. WO 92/12245); affinity chromatography by immobilizing the FGF mutein measuring the binding of labeled or unlabeled heparin or by calculating a thermodynamic dissociation constant for heparin affinity for each FGF mutein (e.g., see European patent application Publication No. EP 0 645 451).

Using such assays, the relative affinities of the FGF muteins for FGF receptors and heparin have been and can be assessed. Those that possess the desired in vitro properties, such as significantly reduced FGF receptor binding affinity for one or more FGF receptor and normal heparin binding activity, are selected. The selected FGF muteins that exhibit desirable activities, e.g., specifically bind to heparin but do not bind to their cognate receptor, may be therapeutically useful in the methods described herein and are tested for such uses employing the above-described assays from which the in vivo effectiveness may be evaluated [Gospodarowicz *et al.* (1987) Endocrin. Rev. 8:95-114; Buntrock *et al.* (1982) Exp. Pathol. 21:62-67; International Patent Application Publication No WO 92/08473]. FGF muteins that exhibit the in vitro activities that correlate with the in vivo effectiveness will then be formulated in suitable pharmaceutical compositions and used as therapeutics.

E. Formulation of pharmaceutical compositions

Compositions are provided for use in the methods herein that contain therapeutically effective amounts of an FGF mutein or peptide-encoding fragment thereof. The FGF mutein are preferably formulated into suitable pharmaceutical preparations such as tablets, capsules or elixirs, for oral administration or in sterile solutions or suspensions for parenteral or intravenous or intramuscular administration. They may also be provided in transdermal patches. Typically the FGF muteins described

above are formulated into pharmaceutical compositions using techniques and procedures well known in the art.

A suitable amount, preferably about 10 to 500 mg of an FGF mutein or mixture of FGF muteins or a physiologically acceptable salt thereof is compounded with a physiologically acceptable vehicle, carrier, excipient, binder, preservative, stabilizer, flavor, etc., in a unit dosage form as called for by accepted pharmaceutical practice. The amount of active substance in those compositions or preparations is such that a suitable dosage in the range indicated is obtained. The precise dosage may be determined empirically.

To prepare compositions, one or more FGF mutein is mixed with a suitable pharmaceutically acceptable carrier. Upon mixing or addition of the FGF mutein(s), the resulting mixture may be a solution, suspension, emulsion or the like. Liposomal suspensions may also be suitable as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art. The form of the resulting mixture depends upon a number of factors, including the intended mode of administration and the solubility of the FGF mutein in the selected carrier or vehicle. The effective concentration is sufficient for ameliorating the symptoms of the disease, disorder or condition treated and may be empirically determined.

Pharmaceutical carriers or vehicles suitable for administration of the FGF muteins provided herein include any such carriers known to those skilled in the art to be suitable for the particular mode of administration. In addition, the active materials can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action or have other action. The FGF muteins may be formulated as the sole pharmaceutically active

BNSDOCID <WO 9955861A2 I >

ingredient in the composition or may be combined with other active ingredients.

In instances in which the FGF muteins exhibit insufficient solubility, methods for solubilizing compounds may be used. Such methods are known to those of skill in this art, and include, but are not limited to, using cosolvents, such as dimethylsulfoxide (DMSO), using surfactants, such as tween, or dissolution in aqueous sodium bicarbonate. Derivatives of the compounds, such as salts of the compounds or prodrugs of the compounds may also be used in formulating effective pharmaceutical compositions.

The concentrations or FGF muteins are effective for delivery of an amount, upon administration, that ameliorates the symptoms of the disorder for which the FGF muteins are administered. Typically, the compositions are formulated for single dosage administration.

The FGF muteins may be prepared with carriers that protect them against rapid elimination from the body, such as time release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, microencapsulated delivery systems.

The FGF mutein is included in the pharmaceutically acceptable carrier in an amount sufficient to exert a therapeutically useful effect in the absence of undesirable side effects on the patient treated. The therapeutically effective concentration may be determined empirically by testing the activity of the FGF muteins in known in vitro and in vivo model systems for the treated disorder.

The compositions can be enclosed in ampules, disposable syringes or multiple or single dose vials made of glass, plastic or other suitable material. Such enclosed compositions can be provided in kits.

The concentration of FGF mutein in the drug composition will depend on absorption, inactivation and excretion rates of the active

compound, the dosage schedule, and amount administered as well as other factors known to those of skill in the art.

The composition may be administered at once, or may be divided into a number of smaller doses to be administered at intervals of time. It is understood that the precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by extrapolation from in vivo or in vitro test data. It is to be noted that concentrations and dosage values may also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed compositions.

If oral administration is desired, the FGF mutein should be provided in a composition that protects it from the acidic environment of the stomach. For example, the composition can be formulated in an enteric coating that maintains its integrity in the stomach and releases the active compound in the intestine. The composition may also be formulated in combination with an antacid or other such ingredient.

Oral compositions will generally include an inert diluent or an edible carrier and may be compressed into tablets or enclosed in gelatin capsules. For the purpose of oral therapeutic administration, the active compound or compounds can be incorporated with excipients and used in the form of tablets, capsules or troches. Pharmaceutically compatible binding agents and adjuvant materials can be included as part of the composition.

The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder, such as, but not limited to, gum tragacanth, acacia, corn starch or gelatin; an excipient such as microcrystalline cellulose, starch and lactose, a disintegrating agent such as, but not limited to, alginic acid and corn starch; a lubricant such as, but not limited to, magnesium stearate; a glidant, such as, but not limited to, colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; and a flavoring agent such as peppermint, methyl salicylate, and fruit flavoring.

When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar and other enteric agents. The compounds can also be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

The FGF muteins or peptides thereof can also be mixed with other active materials, that do not impair the desired action, or with materials that supplement the desired action, including viscoelastic materials, such as hyaluronic acid, which is sold under the trademark HEALON (solution of a high molecular weight (MW of about 3 millions) fraction of sodium hyaluronate; manufactured by Pharmacia, Inc. see, e.g., U.S. Patent Nos. 5,292,362, 5,282,851, 5,273,056, 5,229,127, 4,517,295 and 4,328,803), VISCOAT (fluorine-containing (meth)acrylates, such as, 1H,1H,2H,2H-heptadecafluorodecylmethacrylate; see, e.g., U.S. Patent Nos. 5,278,126, 5,273,751 and 5,214,080; commercially available from Alcon Surgical, Inc.), ORCOLON (see, e.g., U.S. Patent Nos. 5,273,056;

commercially available from Optical Radiation Corporation), methylcellulose, methyl hyaluronate, polyacrylamide and polymethacrylamide (see, e.g., U.S. Patent No. 5,273,751). The viscoelastic materials are present generally in amounts ranging from about 0.5 to 5.0%, preferably 1 to 3% by weight of the conjugate material and serve to coat and protect the treated tissues. The compositions may also include a dye, such as methylene blue or other inert dye, so that the composition can be seen when injected into the eye or contacted with the surgical site during surgery.

Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include any of the following components: a sterile diluent, such as water for injection, saline solution, fixed oil, a naturally occurring vegetable oil like sesame oil, coconut oil, peanut oil, cottonseed oil, etc. or a synthetic fatty vehicle like ethyl oleate or the like, polyethylene glycol, glycerine, propylene glycol or other synthetic solvent; antimicrobial agents, such as benzyl alcohol and methyl parabens; antioxidants, such as ascorbic acid and sodium bisulfite; chelating agents, such as ethylenediaminetetraacetic acid (EDTA); buffers, such as acetates, citrates and phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose. Parental preparations can be enclosed in ampules, disposable syringes or multiple dose vials made of glass, plastic or other suitable material. Buffers, preservatives, antioxidants and the like can be incorporated as required.

The ophthalmologic indications herein are typically treated locally either by the application of drops to the affected tissue(s), contacting with a biocompatible sponge that has absorbed a solution of the FGF muteins or by injection of a composition. For the indications herein, the composition will be applied during or immediately after surgery in order

to prevent closure of the trabeculectomy, prevent a proliferation of keratocytes following excimer laser surgery, prevent the proliferation of lens epithelial cells following cataract surgery or to prevent a recurrence of pterygii. The composition may also be injected into the affected tissue following surgery and applied in drops following surgery until healing is completed. For example, to administer the formulations to the eye, it can be slowly injected into the bulbar conjunctiva of the eye.

If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof. Liposomal suspensions, including tissue-targeted liposomes, may also be suitable as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art. For example, liposome formulations may be prepared as described in U.S. Patent No. 4,522,811.

The active compounds may be prepared with carriers that protect the compound against rapid elimination from the body, such as time release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, implants and microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as collagen, ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid and others. Methods for preparation of such formulations are known to those skilled in the art.

The compounds may be formulated for local or topical application, such as for topical application to the skin and mucous membranes, such as in the eye, in the form of gels, creams, and lotions and for application to the eye or for intracisternal or intraspinal application. Such solutions,

may be formulated as 0.01% - 100% (weight to volume) isotonic solutions, pH about 5-7, with appropriate salts. The compounds may be formulated as aerosols for topical application, such as by inhalation [see, e.g., U.S. Patent Nos. 4,044,126, 4,414,209, and 4,364,923].

Finally, the FGF mutein may be packaged as articles of manufacture containing packaging material, an acceptable composition containing an FGF mutein provided herein, which is effective for treating FGF-mediated disorders, and a label that indicates that the FGF mutein is used for treating FGF-mediated disorders.

F. Methods of treating of FGF-mediated disorders

Methods using FGF mutein and FGF mutein peptide compositions containing therapeutically effective concentrations of the FGF mutein or FGF mutein peptide for treating disorders, particularly proliferative disorders, in which FGF causes or contributes to the pathology are provided herein. In particular, FGF muteins having decreased mitogenic activity compared to wild type, but comparable or increased receptor binding affinity for one or more FGF receptors may be used to prevent the undesired growth and proliferation of FGF-sensitive cells occurring in vascular disorders characterized by accelerated smooth muscle cell proliferation, such as rheumatoid arthritis, tumor angiogenesis, Kaposi's sarcoma, restenosis, In-stent restenosis, certain ophthalmic disorders and dermatological disorders, such as psoriasis, are provided herein.

Preferably, the medicament containing the FGF mutein is administered intravenously (IV), although treatment by localized administration may be tolerated in some instances. Generally, the medicament containing the FGF mutein is injected into the circulatory system of a subject in order to deliver a dose to the targeted cells that express specific FGF receptors, particularly bFGF receptors. Dosages

may be determined empirically, but will typically be in the range of about 0.01 mg to about 100 mg of the compound per kilogram of body weight are expected to be employed as a daily dosage.

Restenosis and vascular injury

Methods for treating vascular injury, particularly, restenosis by contacting the vascular wall with an effective amount of an FGF mutein having reduced biological (i.e. mitogenic) activity are provided.

Atherosclerosis, also referred to as arteriosclerosis, results from the development of an intimal lesion and the subsequent narrowing of the vessel lumen. Frequently, atherosclerosis originally appears as a result of the buildup of plaque which lines the interior of blood vessels, particularly the arteries. Whereas bypass surgery is sometimes employed to replace such clogged arteries, in recent years, a number of surgical procedures have been developed so as to interarterially remove such plaque, often by balloon catheterization or other such treatments in which the plaque is either compressed against or scraped away from the interior surface of the artery. This scraping of the interior wall removes endothelial cells, which constitute the lining of the blood vessel. As a result of this removal, the smooth muscle cells (SMCs), which are normally located exterior of the endothelial cells (ECs) and form the blood vessel structure, begin to grow and multiply causing a narrowing of the vessel lumem. Not infrequently, the patient so treated finds a recurrence of such narrowing of the vessel lumen in a relatively short period thereafter as a result of this proliferation, generally referred to as restenosis, requiring a repetition of the surgical procedure to again remove the increasing blockage. Angioplasty can also result in injury to SMCs.

Proliferating SMCs express functional FGF receptors and are responsive to bFGF. By inhibiting proliferation of migrating smooth

muscle cells (SMCs), it is possible to prevent the undesirable growth and ultimate clogging which occurs following vascular injury, and which is generally referred to as restenosis [e.g., see Kearney *et al.* Circul. 95:1998-2002 (1997)]. Basic FGF appears to play a pivotal role in the subsequent responses of the vascular wall [e.g., see Linder *et al.* Proc. Natl. Acad. Sci. U.S.A. 88:3739-3743 (1991)]. Basic FGF is known to be synthesized by endothelial and smooth muscle cells (SMCs) and is thought to be stored in the subendothelial matrix, and in some instances, this growth factor is released from cells after injury. Therefore, FGF muteins having decreased mitogenic activity compared to wild type, but comparable or increased receptor binding affinity for one or more FGF receptors that inhibit FGF-mediated proliferation of SMCs may be used in methods for treating restenosis by preventing the proliferation that causes the narrowing of the vessel lumen.

Treatment is effected by administering a therapeutically effective amount of a medicament containing the FGF mutein in a physiologically acceptable carrier or recipient, in a manner so that the FGF mutein reaches regions in a human or other mammal where the FGF mutein will inhibit the proliferation of the target cells. For restenosis, intraarterial infusion will be among the preferred methods. Although a single dose should inhibit neointimal proliferation, IV administration over a period of time is preferred.

Compositions containing a therapeutically effective amount of an FGF mutein for treating restenosis and In-Stent restenosis may be formulated for intravenous or local administration. Alternatively, the FGF muteins may be conjugated to an agent that specifically targets proliferating SMCs, such as antibodies, hormones, ligands or the like to improve delivery and uptake of the compound. The therapeutically effective concentration may be determined empirically by testing the

compounds in known in vitro and in vivo systems (see, e.g., Mostacelli *et al.* (1987) J. Cell. Physiol. 131:123-130]; mitogenic assays [Gospardarowicz *et al.* (1984) Proc. Natl. Acad. Sci. U.S.A. 81:6963-6967; Thomas *et al.* (1984) Proc. Natl. Acad. Sci. U.S.A. 81:357]; stimulation of angiogenesis in vitro [see, e.g., European Patent Application No. EP 645 451]; cell proliferation assays or heparin binding assays [see, e.g., International Application Publication No. WO 92/12245]; assays measuring the release of cellular proteases [Mostacelli *et al.* (1986) Proc. Natl. Acad. Sci. U.S.A. 83:2091-2095; Phadke (1987) Biochem. Biophys. Res. Comm. 142:448-453]; and, assays for the promotion of FGF-mediated neurite outgrowth and neuron survival [Togari *et al.* (1983) Biochem. Biophys. Res. Comm. 114:1189-1193; Wagner *et al.* (1986) J. Cell Biol. 103:1363-1367]) and then extrapolated therefrom for dosages for humans.

Rheumatoid arthritis

Rheumatoid arthritis is a systemic, chronic inflammatory disease, that is characterized by the destruction of the joint cartilage and inflammation of the synovium. The hallmark feature of rheumatoid arthritis is the production circulating autoantibodies, also referred to as rheumatoid factors, which are reactive with the Fc portions of the patient's IgG molecules [e.g., see Abbas *et al.*, Cellular and Molecular Immunology, W.B. Saunders Co., Philadelphia, PA].

One of the systemic complications of rheumatoid arthritis is the formation of injurious immune complexes in the synovial fluid of the joints that initiates vascular inflammation by activation of the complement cascade. T-cells, activated B-cells, plasma cells and

macrophages are often found in synovial fluid of affected joints as well as a variety of soluble proteins, such as cytokines [e.g., interleukin-1, IFN-γ and tumor necrosis factor (TNF)] and growth factors, such as bFGF. It has been suggested that cytokines act in concert with the inflammatory mediators, e.g., bFGF, to cause local tissue destruction. Chronically, cytokines and bFGF stimulate fibroblast and collagen proliferation resulting in angiogenesis, and prolonged exposure can result in hyperproliferation of epithelial cells that form fibrous tissue, referred to as fibrosis.

Thus, FGF muteins having decreased mitogenic activity compared to wild type, but comparable or increased receptor binding affinity for one or more FGF receptors that inhibit the FGF-mediated hyperproliferation of epithelial cells, such as those corresponding to Leu138 of bFGF, may be used to treat rheumatoid arthritis. The FGF muteins for treating rheumatoid arthritis may be formulated for oral administration or intravenous injection and an effective concentration may be administered. The effective concentration is sufficient for ameliorating the symptoms of the disease, disorder or condition treated and may be empirically determined.

Tumor Angiogenesis

Angiogenesis plays a critical role in embryonic development and in several physiologic and pathologic conditions, including wound healing, ovulation, diabetic retinopathy and malignancy. In particular, without the nutrients and oxygen provided via this neovascularization, solid tumors would be unable to grow beyond about 2 mm in diameter.

Evidence exists that several neoplaisas, including melanomas, ovarian, pancreatic and some colon carcinomas, have receptors for bFGF. Testing with radioactive binding assays on a number of human carcinogenic cell lines isolated from human cancers demonstrated that

BNSDOCID <WO 9955861A2 I >

many but not all of these cell lines bind ^{125}I-FGF. Tumor growth may be inhibited by modulating FGF receptor activity in the components of a blood vessel, e.g., vascular endothelial cells or vascular SMCs [see e.g., Haberman Angiogenesis :98-1-98-20 (1996); Coville-Nash *et al.* Molec. Med. Today :14-23 (1997); Shawver *et al.* Drug Discov. Today 2:50-63]. Thus, FGF muteins having decreased mitogenic activity compared to wild type, but comparable or increased receptor binding affinity for one or more FGF receptors (e.g., FGF muteins corresponding to Leu138 of bFGF) that inhibit the activity of FGF may be used to treat tumorigenic pathophysiological conditions caused by a proliferation of cells which are sensitive to FGF mitogenic stimulation.

The FGF muteins may be specifically targeted to tumorigenic tissues by direct interaction with its receptor, by linking the FGF mutein to an agent that specifically binds to the surface of the tumorigenic cell, e.g., an anti-tumor antigen antibody, or linking the FGF mutein to an agent that is preferentially interacts with or taken up by targeted tumor. In addition, FGF muteins may be encapsulated in tissue-targeted liposomal suspensions for targeted delivery of the compound.

The FGF muteins for treating tumor angiogenesis may be formulated for topical application and administered to the skin, e.g., for treatment of melanoma, or may be formulated for intravenous administration for treatment of solid tumors, such as carcinomas. The therapeutically effective concentration may be determined empirically by testing the FGF muteins in known in vitro assays, e.g., inhibition of angiogenesis in vitro (see, e.g., European Patent Application No. EP 645 451]) and then extrapolated therefrom for dosages for humans.

Ophthalmic Disorders

Pharmaceutical compositions containing a therapeutically effective amount of an FGF muteins having decreased mitogenic activity compared to wild type, but comparable or increased receptor binding affinity for one or more FGF receptors may be used in methods of treating ophthalmic disorders resulting from FGF-mediated hyper-proliferation of lens epithelial cells, fibroblasts or keratinocytes [e.g., see Dell Drug Discov. Today 1:221-222 (1996)]. In particular, ophthalmic disorders that may be treated using the methods and compositions provided herein include, but are not limited to, diabetic retinopathy, corneal clouding following excimer laser surgery, closure of trabeculectomies, hyperproliferation of lens epithelial cells following cataract surgery and the recurrence of pterygii.

The FGF mutein compositions for treating ophthalmic disorders may be formulated for local or topical application and administered by topical application of an effective concentration to the skin and mucous membranes, such as in the eye. The compositions may also include a dye, such as methylene blue or other inert dye, so that the composition can be seen when injected into the eye or contacted with the surgical site during surgery. The effective concentration is sufficient for ameliorating the symptoms of the disease, disorder or condition treated and may be empirically determined.

In addition, pharmaceutical compositions containing a therapeutically effective amount of an FGF mutein corresponding to positions Val88 and Phe93 of bFGF that specifically bind to heparin but have reduced FGF receptor binding affinity may be used to treat the ophthalmic disorders resulting from heparin potentiation of FGF-mediated hyper-proliferation of lens epithelial cells, fibroblasts or keratinocytes. In particular, ophthalmic disorders that may be treated using the

compositions provided herein include, but are not limited to, diabetic retinopathy, corneal clouding following excimer laser surgery, closure of trabeculectomies, hyperproliferation of lens epithelial cells following cataract surgery and the recurrence of pterygii.

The ophthamologically acceptable compositions are applied to the affected area of the eye. For treatment of corneal clouding the composition is applied during or immediately after surgery. In particular, following excimer laser surgery, the composition is applied to the cornea; following trabeculectomy the composition is applied to the fistula; and following removal of pterygii the composition is applied to the cornea.

The compositions can be applied as drops for topical and subconjunctival application can be injected into the eye for intraocular application. The compositions may also be absorbed to a biocompatible support, such as a cellulosic sponge or other polymer delivery device, and contacted with the affected area.

G. Methods of treating heparin-related disorders

Methods using FGF mutein and FGF mutein peptide compositions containing therapeutically effective concentrations of the FGF mutein for treating disorders, particularly disorders associated with the systemic administration of heparin, in which heparin causes or contributes to the pathology are provided herein. In particular, FGF muteins corresponding to positions Val88 and Phe93 of bFGF that specifically bind to heparin but have reduced FGF receptor binding affinity may be used to prevent excessive bleeding resulting from the anti-coagulant activity of heparin, heparin-induced thrombosis and thrombocytopenia and to prevent the potentiation of undesired growth and proliferation of FGF-sensitive cells occurring in angiogenesis and ophthalmic disorders, are provided herein.

In certain embodiments, the methods of treating heparin-related disorders use the FGF mutein compositions and pharmaceutical

compositions in which the residues corresponding to Val88 or Phe93 of bFGF; whereas in other embodiments the methods use the Val88 or Phe93 FGF muteins that have further amino acid replacements that decrease receptor binding but retain a high affinity for heparin (e.g., amino acid substitutions corresponding to residues Glu^{96} and Leu^{140} of bFGF; Springer *et al.* (1994) J. Biol. Chem. 269: 26879-26884; Zhu *et al.* (1995) J. Biol. Chem. 270: 10222-10230.

Heparin-induced thrombosis and thrombocytopenia

As noted above, heparin is a widely used adjunctive agent for acute management of thrombosis and is a treatment of choice for preventing and treating venous thromboembolism. Although heparin is widely used as the injectable anticoagulant of choice, it has several potential shortcomings. For example, the systemic administration of high levels of heparin used to impede local thrombus deposition also can results in the global reduction in Factor Xa and/or Factor IIa activity. Thus, a complication of systemic heparin therapy is severe bleeding in patients because of the reduced capability of blood to coagulate (e.g., Visentin *et al.* (1995) Curr. Opin. Hematol. 2:351-357). Severe bleeding is a serious thromboembolic complication of heparin therapy and can result in crippling disabilities and/or death (e.g., see Sodian *et al.* (1997) ASAIO J. 43:M430-M433). A notorious complication of systemic heparin therapy is heparin-induced thrombocytopenia. Heparin-induced thrombocytopenia (HIT) is an immunoglobulin-mediated adverse drug reaction associated with a high risk of thrombotic complications.

Methods of treating heparin-induced and heparin-related disorders such excessive bleeding in patients that arise from the anticoagulant activity of heparin and methods of treating thrombocytopenia and thrombosis by administering a therapeutically effective amount of FGF mutein having amino acid substitutions at positions corresponding to

BNSDOCID <WO 9955861A2 I >

Val88 or Phe93 of bFGF that binds to heparin but has significantly reduced receptor binding activity are provided. The FGF muteins may also have further amino acid substitutions that effect FGF receptor binding activity (e.g., those corresponding to Glu96 of bFGF).

Preferably, the medicament containing the Val88 or Phe93 FGF mutein is administered intravenously (IV), although treatment by localized administration of the composition may be tolerated in some instances. Generally, the medicament containing the FGF mutein is injected into the circulatory system of a subject in order to deliver a dose to bind the desired amount of heparin. Alternatively, the FGF mutein can be formulated for topical or local administration and applied at the desired location (i.e., at a wound). Dosages may be determined empirically, but will typically be in the range of about 0.01 mg to about 100 mg of the compound per kilogram of body weight are expected to be employed as a daily dosage.

The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

EXAMPLE 1

A. Materials and Methods

Materials

A human synthetic bFGF gene was purchased from R and D Systems (Minneapolis, MN). Expression vector pET11d and bacterial strain BL21(DE3) were obtained from Novagen (Madison, WI). Baculovirus transfection vector PVL1393 was obtained from PharMingen (San Diego, CA). A Magic Mini preparation kit was obtained from Promega (Madison, WI). Heparin-Sepharose was obtained from Pharmacia-LKB Biotechnology (Uppsala, Sweden). Heparin was purchased from Sigma (St Louis, MO). FGFR1β-TPA fusion protein was a gift from Eisai (Tsukuba, Japan). [^{125}I]bFGF was obtained from NEN

Research Products. Anti-bFGF monoclonal antibody was purchased from Upstate Biotechnology. Alkaline phosphatase-conjugated anti-mouse I_BG antibodies were purchased from Bio-Rad. Prestained protein molecular weight standards were purchased from GIBCO/BRL. All other chemicals were of reagent grade, purchased from Sigma.

Identification of residues for mutagenesis

The crystal structure of bFGF was obtained from the protein data bank (code 3FGF; e.g., see Abola *et al.* (1987) in Crystallographic Databases-Information Content, Software Systems, Scientific Applications, Allen *et al.* eds., Data Commission of the International Union of Crystallography, Cambridge, pp. 107-132; Koetzle *et al.* (1977) J. Mol. Biol. 112:535-542) and analyzed computationally as described previously (Zhu *et al.* (1995)J. Biol. Chem. 270:21869-21874). Briefly, hydrogen atoms were added to the initial coordinates, and minimized the system to relieve repulsive stearic interactions. To evaluate solvent effects on the protein, its geometric center was determined and a spherical shell of water of 26 Å radius was placed around it to completely immerse it in a spherical water bath. The total of the solvated protein system was 5444 atoms. A dielectric constant of 4.0 was used in the calculations. Three stages of minimization were carried out on the system prior to the dynamics simulations. Initially, only the solvent around the protein was minimized for 500 cycles to optimize the interactions among the solvent molecules. In the second stage, 500 iterations of minimization were carried out for the total solvent/protein to optimize the interaction between the solvent and protein. Finally, the whole system was minimized again with the SHAKE option (Ryckert *et al.* (1977) J. Comput. Phys. 23:327-334), to constrain the bonds in the system.

The molecular dynamics simulation was carried out at 300 K and 1 atm pressure. After 500 picoseconds of equilibration, data were collected every picosecond over a period of 50 picoseconds. Each conformer obtained at 25 picosecond intervals was minimized and stored for further analysis. The computations were performed using an extensively modified version (Ramnarayan *et al.* (1990) J. Chem. phys. 92:7057-7067) of the AMBER program (Singh *et al.* (1986) AMBER 3.0, University of Calfornia, San Franscisco). The time averaged conformations resulting from the molecular dynamics calculations were analyzed to derive information regarding residues that form previously unidentified hydrophobic patches for the site-directed mutagenesis studies.

B. Mutagenesis, protein expression and purification

The construction of the human bFGF gene into the pET11d vector, mutagenesis and expression and purification are described below. Briefly, after site-directed mutagenesis, the expression vector was transformed into the BL2I(DE3) *Escherichia coli* strain. Cultures were grown to an A_{600} of 0.8 in LB medium containing 40 μg/ml ampicillin at 37°C. Expression of bFGF and muteins was induced by adding 0.4 mM isopropyl-β-D-thiogalactopyranoside and the cultures were further grown for 3 h. The bFGF was purified using a CM-Sepharose column, followed by a heparin-Sepharose column. The concentration of wild-type bFGF and its mutants was then determined.

C. Preparation of mutagenized FGF peptides by site-directed mutagenesis

Site-directed mutagenesis was and can be performed using a commercially available site-directed mutagenesis kit [Clontech, Palo Alto, CA] according to the instructions provided by the manufacturer.

Plasmid isolation, production of competent cells and transformation were carried out according to published procedures (Sambrook et al. (1989) *Molecular Cloning, a Laboratory Manual Cold*, Spring Harbor Laboratory Press, Cold Spring Harbor, NY). Purification of DNA fragments was achieved using the Magic mini-prep kit, purchased from Promega, (Madison, WI).

1. Mutagenesis of bFGF

A synthetic DNA encoding human bFGF [SEQ ID NO:13; commercially available from R & D Systems, Minneapolis, MN] containing a 5' *Nco*I and 3' *Bam*HI overhangs was ligated into the *Nco*I and *Bam*HI sites of pET11d. The bFGF-pET11d DNA template was denatured in an excess of two complementary primers: a bFGF-specific primer containing the desired substitutions in the bFGF coding region; and a *Bam*HI selection primer provided by the manufacturer. The *Bam*HI specific primer introduces a mutation into the resulting plasmid that inactivates the *Bam*HI site in the multiple cloning site thereby allowing for enrichment of mutagenized plasmids during transformation using *Bam*HI.

Oligonucleotide primers used for site-directed mutagenesis of human bFGF were synthesized based on the reported bFGF sequence (SEQ ID NO:2) except for nucleotide substitutions in the coding region at amino acid positions Val88, Phe93 and Leu138. The two primers were annealed to the denatured template by slow cooling, followed by in vitro second strand synthesis and ligation. Unmutagenized vector DNA was digested with *Bam*HI and a portion of the partially digested ligation mixture was used to transform competent E. coli mutS strain BMH 71-18, which was provided by the manufacturer. Plasmid DNA was purified from the resulting Amp^R transformants using a Magic mini-prep kit [Promega, Madison, WI] and plasmid DNA isolated from single colony

BNSDOCID <WO 9955861A2 I >

transformants was sequenced to verify the presence of each bFGF mutation.

2. Recombinant expression and purification of mutagenized bFGF

Plasmids encoding bFGF muteins were transformed into the E. coli strain BL21(DE3) [Novagen, Madison, WI], which contains a copy of the T7 RNA polymerase gene under the control of the lacUV5 operon promoter. Transformants were selected for resistance to ampicillin and the cells from single colony transformants were grown at 37°C to mid-log phase (A_{600} = 0.8) in LB medium [Sambrook *et al.*, 1989] supplemented with 40 μg/ml ampicillin. Recombinant expression of FGF muteins was induced by the addition of 0.4 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and expression was allowed to proceed for an additional 4 hours at 37°C.

Cells were pelleted by centrifugation, lysed by standard procedures (e.g., see Seno *et al.* (1990) Eur. J. Biochem. 188:239-245) and the cellular debris was removed by centrifugation. The cytoplasmic fraction containing the soluble FGF muteins was loaded onto a carboxymethyl-Sepharose (CM-Sepharose) column (e.g., Pharmacia) and the bound bFGF muteins were eluted from the column using a high salt gradient (e.g., NaCl or NH_4OAc). The bFGF mutein-containing fractions were pooled, dialyzed against buffer A [25 mM Tris-HCl, pH 7.5; 0.6 M NaCl] and loaded onto a heparin-Sepharose column (Pharmacia) equilibrated in buffer A. The column was washed extensively with buffer B (buffer A supplemented to 1.0 M NaCl), and bound FGF muteins were eluted from the column by the addition of buffer C (buffer A supplemented to 2.0 M NaCl).

Samples of the purified protein fractions were subject to electrophoresis on 12% SDS-polyacrylamide gels and resolved proteins

were visualized by staining with Coomassie Blue 250. The concentration and purity of the various FGF muteins were determined using a scanning laser densitometry and bovine serum albumin as a standard or by using a commercially available kit based on the method of Bradford [e.g., Bio-Rad].

EXAMPLE 2

Assays for measuring the binding FGF muteins to an FGF receptor

A. Soluble FGF receptor assay

The binding activity of the FGF muteins for one or more FGF receptor was and can be determined by testing the ability of an FGF mutein to compete with ^{125}I-bFGF for binding to one or more FGF receptor or FGF-binding fragment thereof. In one embodiment, a recombinant FGF receptor fusion protein was used in which the extracellular domain of a human FGF receptor, FGFR1, was fused to the amino terminal fragment of tissue plasminogen activator (tPA) protein. This fusion protein retains the ability to bind FGF, such as bFGF [Zhu *et al.* (1995) J. Biol. Chem. 270:21869-21874].

(i) Isolation of DNA encoding the shorter form of human fibroblast growth factor receptor 1 (FGFR1)

The nucleotide sequence of the DNA encoding the shorter form of human basic fibroblast growth factor receptor 1 (FGFR1) has been determined [e.g., N. Itoh *et al.*, (1990) Biochem. Biophys. Res. Comm 169:680-685]. This shorter form of FGFR1 is a 731 amino acid polypeptide that has a signal peptide, two extracellular immunoglobulin-like domains, a transmembrane domain and an intracellular tyrosine kinase domain.

Based on the reported sequence, two oligonucleotides complementary to sequences flanking the FGFR1 coding region were synthesized and used as primers in polymerase chain reactions (PCR) to

BNSDOCID <WO 9955861A2 I >

isolate a DNA encoding a full-length human FGFR1 from a human aorta cDNA library (Quickclone, Clontech, Palo Alto, CA). PCR amplification was performed using a commercially available PCR kit according to manufacturer's instructions (Perkin Elmer Cetus, Norwalk, CT). An oligonucleotide corresponding to nt -20 to +5, relative to the A of the ATG initiation codon of FGFR1, [e.g., N. Itoh *et al.*, (1990) Biochem. Biophys. Res. Comm. 169:680-685] and an oligonucleotide complementary to nt 2218-2243 were used as primers to amplify a 2,243 bp PCR product encoding the entire FGFR1 coding region.

The full-length FGFR1-encoding DNA was used as a template for a subsequent PCR reaction, performed as described above, to amplify a 869 bp DNA fragment encoding only the FGFR1 extracellular domain. Simultaneously, a HindIII restriction endonuclease site was introduced upstream of the FGFR1 initiation codon and a SalI site was introduced downstream of the second immunoglobulin-like extracellular domain (IgII) to facilitate cloning of the amplified product.

The HindIII site was introduced at nt -8 to -3 during the PCR reaction by synthesizing an oligonucleotide primer corresponding to nt -12 to +22 that introduced nucleotide changes at three positions in the FGFR1 sequence: nt -3 (G to T), nt -6 (A to G) and nt -8 (G to A). The SalI site was introduced at nt 849 to nt 854 by synthesizing an oligonucleotide primer complementary to nt 823 to 857 containing nucleotide substitutions at three positions in the FGFR1 sequence: nt 849 (C to G), nt 851 (G to C) and nt 854 (G to C). The 857 bp PCR fragment was incubated with HindIII and SalI and purified by agarose gel electrophoresis according to the standard procedures [Sambrook *et al.*, (1989) Molecular Cloning, 2nd ed., Cold Spring Harbor Laboratory Press, New York]. The DNA was isolated from gel by electroelution and recovered by precipitation with ethanol.

Thus, the resulting HindIII to SalI DNA fragment consists of nt -7 to nt 849 of the FGFR1 cDNA described by Itoh *et al.* and encodes amino acid residues 1 to 284 of the shorter form of the bFGF receptor.

(ii) Isolation of DNA encoding human tissue plasminogen activator

The nucleotide sequence of the DNA encoding human tissue plasminogen activator (tPA) has been determined [e.g., see Pennica *et al.* (1983) Nature 301:214-221]. Human tPA is a 562 amino acid polypeptide which is processed during secretion to its mature form by cleavage of a 35 amino acid signal peptide. Several regions of the primary structure of mature tPA have a high degree of homology to known structural domains of other proteins, such as homology to the finger and growth factor domains, the Kringle 1 and Kringle 2 domains of plasminogen and prothrombin and the C-terminal serine protease domain [e.g., see Ny *et al.* Proc. Natl. Acad. Sci. U.S.A. 81:5355-5359].

Based on the reported sequence, oligonucleotides complementary to sequences flanking the tPA coding region were synthesized and used as primers in PCR reactions to isolate a full-length cDNA encoding human tPA from a human placenta cDNA library (Clontech, Palo Alto, CA). An oligonucleotide corresponding to nt -6 to +21, relative to the A of the initiation codon of the human tPA prepro polypeptide [e.g., see Pennica *et al.* (1983) Nature 301:214-221] and an oligonucleotide complementary to nt 1558 to nt 1584 were used to amplify a 1591 bp DNA encoding the entire human tPA prepro polypeptide.

The full-length DNA was used as a template for a subsequent PCR reaction to amplify a 599 bp DNA encoding a portion of the signal peptide-finger-growth factor-first Kringle domains of tPA, and which also to introduce an in-frame amber stop codon [i.e., UGA] at amino acid codon 180 of mature tPA sequence. Concurrently, a SalI restriction

endonuclease site and a mutation substituting a Pro for an Arg at position -6 were introduced upstream of the first Ser codon of mature tPA and a BamHI site was introduced downstream of newly introduced translational stop codon to allow for convenient subcloning of the amplified product. The substitution of Pro for Arg at amino acid residue position -6 introduces a proteolytic cleavage site for thrombin in the linker sequence (i.e., Phe-Pro-Arg-Gly at positions -7 to -4).

The SalI site and the amino acid substitution were introduced at nt 76 to 81 and 91 and 92 (nt -30 to -25 and -15 and -14, respectively, relative to the first nucleotide of mature tPA) during the PCR reaction by synthesizing an oligonucleotide primer corresponding to nt 72 to nt 111 containing nucleotide substitutions at six positions in the tPA sequence: nt 76 (A to G), nt 79 (C to G), nt 81 (T to C), nt 91 (A to C) and nt 92 (G to C). The BamHI site at nt 652 to nt 657 and translational stop codon at amino acid codon 180 (nt 642-644) were introduced by synthesizing an oligonucleotide primer complementary to nt 623 to 661 containing nucleotide substitutions at three positions in the tPA sequence: nt 644 (C to A), nt 655 (A to T) and nt 657 (G to C).

The amplified PCR fragment was incubated with SalI and BamHI and subjected to agarose gel electrophoresis according to the standard procedures [Sambrook *et al.*, (1989) Molecular Cloning, 2nd ed., Cold Spring Harbor Laboratory Press, New York]. The 585 bp DNA was isolated from gel by electroelution and recovered by precipitation with ethanol.

(iii) Construction of a vector for expressing human FGFR1-tPA fusion protein

The isolated SalI to BamHI fragment encoding the portion of human tPA was ligated into the SalI and BamHI sites of pUC18 to generate plasmid HTPA3/4-pUC18. HTPA3/4-pUC18 was then digested

with HindIII and SalI into which the isolated HindIII to SalI FGFR1-encoding fragment was inserted. The plasmid carrying the FGFR1-tPA chimeric DNA was digested with HindIII and BamHI, subjected to agarose gel electrophoresis and the 1,426 bp DNA fragment was excised from the gel and isolated as described above. The resulting DNA encodes a 472 amino acid peptide comprised of amino acids 1-284 of human FGFR1, a 10 amino acid linker sequence VDARFPRGAR, derived from the human tPA signal peptide, and amino acids 1-178 from human tPA. The resulting DNA encoding the FGFR1-tPA fusion protein is shown in SEQ ID No: 11 and the deduced amino acid is shown in SEQ ID No: 12.

The DNA of SEQ ID No. 11 was digested with HindIII to BamHI and the 1,434 bp fragment (nt 2-1435 of SEQ ID No: 11) was isolated and ligated into the mammalian expression vector pK4K for recombinant expression of the FGFR1-tPA fusion protein (Niidome, T. *et al.* (1994) Biochem. Biophys. Res. Commun. 203, 1821-1827). The plasmid pK4K is a pBR322-based vector that has unique HindIII and BamHI sites for directional cloning of heterologous DNAs whose expression is under the control of the SV40 early promoter. This plasmid also contains the β-lactamase and DHFR genes for use as selectable markers in prokaryotes and eukaryotic organisms, respectively.

(iv) Expression of FGFR1-tPA chimeric protein in mammalian cells

Baby hamster kidney cells (BHK cells; Waechter, D.E., *et al.* (1982) Proc. Natl. Acad. Sci., USA:79:1106) were transfected with 5 μg of the FGFR1-tPA-containing expression plasmid using the CellPhect calcium phosphate method according to manufacturer's instructions (Pharmacia, Sweden). Transfectants were selected for the presence of the DHFR gene by selecting resistance to methotrexate and maintained in

Dulbecco's Eagle medium containing 10% fetal bovine serum and 250 nM methotrexate.

Upon expression, the recombinant FGFR1-tPA fusion protein is secreted into the surrounding culture medium. Recombinant FGFR1-tPA fusion protein expression in BHK cells was monitored by sandwich enzyme-linked immunosorbent assays (sandwich ELISAs). A mouse IgG monoclonal antibody specific for human tPA, designated 14-6, was used as the capture antibody and a polyclonal, rabbit anti-IgG antibody conjugated to horseradish peroxidase was used as the secondary-labelled antibody.

(v) Purification of FGFR1-tPA chimeric protein

The recombinant FGFR1-tPA fusion protein was purified from condition medium of BHK-expressing cells by affinity chromatography. Transfected cells were grown as described above and the condition medium was harvested. The osmolarity of the conditioned medium was adjusted to a final concentration of 0.5 M NaCl by the addition of solid NaCl. The sample was applied onto a column of Cellulofine (Seikagaku Kogyo, Tokyo, Japan) conjugated with anti-tPA 14-6 monoclonal antibody previously equilibrated in column buffer [50 mM Tris-HCl, pH 7.5, and 0.5 M NaCl]. The column was then washed with 10 column volumes of column buffer and bound fusion protein was eluted from the column by the addition of 0.2 M glycine-HCl, pH 2.5. Fractions (0.5 ml) were collected into a tube containing 0.5 ml of 1 M Tris-HCl, pH 8.0 to neutralize the acidic eluate. Eluted fractions were monitored for the presence of FGFR1-tPA protein by measuring the absorbance of each fraction at 280 nm. The FGFR1-tPA-containing fractions were dialyzed against PBS and concentrated to a final concentration of 1.5-2.0 mg/ml using Centriprep filters (AMICON).

(vi) Analysis of bFGF-FGFR1 interaction

The soluble, recombinant FGFR1-tPA fusion protein was immobilized to a solid support by attachment to the surface of the wells of an enzyme-linked immunosorborbent assay plate (High binding plates, COSTAR). A 0.1 ml aliquot of a 10 μg/ml solution of rFGFR1-tPA in PBS was added and the plate was incubated for approximately 16 hr at 4°C. Unbound fusion protein was removed by washing three times with an equal volume of cold PBS.

To each well, a 0.1 ml aliquot of blocking buffer (25 mM HEPES, pH 7.5, 100 mM NaCl and 0.5% gelatin) was added, and the samples incubated for 1 hr at ambient temperature to prevent non-specific binding of reagents. The wells were washed three times with binding buffer (25 mM HEPES, pH 7.5, 100 mM NaCl and 0.3 % gelatin) followed by addition of 0.1 ml of binding buffer supplemented with 2 μg/ml heparan sulfate and a range of 1-20ng/ml of labelled ^{125}I-bFGF (800-1200Ci/mmol; Amersham, Arlington Heights, IL) and incubated in the absence or presence of 2.5 μg/ml unlabelled bFGF or varying concentrations of an FGF mutein for 3 hr at ambient temperature. The buffer was removed by aspiration and the wells were washed twice each with PBS and a solution of 25 mM HEPES, pH 7.5, containing 2 M NaCl. Bound bFGF was dissociated from the immobilized fusion protein by the addition of two aliquots of a solution of 25 mM sodium acetate, pH 4.0, containing 2 M NaCl. The two sodium acetate washes were combined and the amount of radioactivity present was determined using a gamma counter.

The amount of bound radiolabelled bFGF in each well was calculated and the specificity of bFGF binding was analyzed according to Scatchard [Scatchard (1949) Ann. N.Y. Acad. Sci. 51:660]. From this analysis, a 280 pM dissociation constant (K_D) for the binding of bFGF to

the recombinant FGFR1-tPA fusion protein was calculated. This value correlates well with 130 pM K_D value reported for bFGF binding to native FGFR1 receptors expressed in smooth muscle cells [Saltis *et al.* (1995) Arteriosclerosis 118:77-87].

B. Membrane-bound FGF receptor assays

(i) Competitive inhibition of FGF binding

The rat aortic smooth muscle cell line, Rb-1, expresses high and low affinity FGF receptors [e.g., see Nachtigal *et al.* (1989) In Vitro Cell. & Develop. Biol. 25:892-897]. The binding activity of the FGF muteins was and can also be determined by the ability of an FGF mutein to compete with ^{125}I-bFGF for binding to the FGF receptors expressed on cell surface of such cells [e.g., see, Mostacelli *et al.* (1987) J. Cell. Physiol. 131:123-130].

Rb-1 cells were grown in 24-well plates to near-confluence in Dulbecco's modified Eagle's medium (DMEM; GIBCO BRL) supplemented with 10% fetal bovine serum, penicillin (100 unit/ml) and streptomycin (100 ug/ml). The culture medium was removed by aspiration and the cells were incubated in binding buffer [serum-free DMEM supplemented with 20 mM HEPES (pH 7.5) and 0.1% BSA] containing 2 ng/ml recombinant human ^{125}I-bFGF (800-1200 Ci/mmol; Amersham, Arlington Heights, IL) and varying concentrations of test compound, for 2 hr at ambient temperature. The nonspecific binding of iodinated bFGF to Rb-1 cells was estimated in parallel reactions performed in the presence of an excess of unlabeled bFGF.

The cells were washed twice with cold phosphate-buffered saline (PBS) and the bFGF bound to low affinity heparan sulfate proteoglycan (HSPG) receptors was dissociated by the addition to each well of a 1 ml solution of 25 mM HEPES (pH 7.5) containing 2 M NaCl. Following removal of the low affinity sample, the bFGF bound to high affinity FGF

receptors was dissociated by the addition to each well of a 1ml solution of 25 mM sodium acetate (pH 4.0) containing 2 M NaCl. A 1 ml aliquot from each well was transferred to a polypropylene tube and the amount of radioactivity present in the low and affinity samples was determined using a gamma counter.

(ii) Competitive inhibition of EGF binding

The specificity of the FGF muteins was and can be examined by measuring the ability of compounds to inhibit the binding of epidermal growth factor (EGF) to the surface of Rb-1 cells. Rb-1 cells were grown as described above and incubated in binding buffer containing 2 ng/ml of ^{125}I-EGF (>750Ci/mmol; Amersham) under similar conditions. Non-specific binding of radiolabelled EGF was estimated in parallel reactions performed in an excess of unlabeled EGF.

After washing the cells twice with cold PBS, specifically bound EGF was dissociated from the cells by addition of a solution of 0.1% Triton-X-100 and 5 min incubation at ambient temperature. The amount of radioactivity in each supernatant was measured using a gamma counter.

C. Inhibition of ^{3}H-thymidine incorporation

The incorporation of radiolabelled nucleotides into newly synthesized cellular DNA may be used as an indicator of cell proliferation. SMCs, such as rat aortic Rb-1 cells, incorporate tritiated thymidine into DNA upon stimulation with bFGF, PDGF or EGF.

The activity of FGF muteins can be assessed by measuring tritiated thymidine incorporation into the DNA of cultured SMCs incubated in the presence of bFGF, PDGF or EGF. An inoculum of approximately 2 X 10^4 Rb-1 cells was added to a plurality of wells and the cells cultured for three days as described in EXAMPLE 2B(i). The cells were washed twice with serum-free medium [DMEM supplemented

with 0.1 % BSA, 5 μg/ml transferrin, 1mM sodium pyruvate, penicillin (100 unit/ml) and streptomycin (100 ug/ml)] and cultured for an additional three days in serum-free DMEM medium.

After washing twice in serum-free DMEM medium, the follow was added to each well: 400 μl of serum-free DMEM, 50 μl of 3 ng/ml of unlabelled bFGF in DMEM and 50 μl of known concentration test compound in DMEM 10% DMSO for 23 hr at 37°C in a 5% CO_2 atmosphere. To each well, 10μl of tritiated thymidine (^{3}H-thymidine, 50 μCi/ml) was added and cells were incubated for 1 hour at 37°C. The medium was removed and the cells were washed twice with cold PBS. An 500 μl aliquot of a cold 10% TCA solution was added to each well and the cells incubated at 4°C overnight. After washing three times in cold PBS, the cells were incubated in 500 μl of 0.5 N NaOH for 30 min and the pH of the sample was neutralized by the addition of an equal volume of 0.5 N HCl. The amount of radioactivity present the supernatant of each well was determined using a liquid scintillation counter.

EXAMPLE 3

Analysis of the bioactivity of FGF muteins

A. FGF receptor binding

Analysis of the molecular structure of bFGF indicates that residues Tyr73, Val88 and Phe93 form a hydrophobic patch on the bFGF surface and are solvent accessible (e.g., see Table 4 below). Based on the structure of bFGF, site-directed mutagenesis was performed to evaluate whether the newly identified hydrophobic patch composed of Tyr73, Val88, Phe93 are required for affinity binding to FGF receptor.

Table 4 shows that substitution of Val88 and Phe93 with alanine reduces the receptor binding affinities -10.4- and 81-fold, respectively,

compared with the wild-type protein, confirming the importance of these residues for high-affinity receptor binding:

Table 4. Water-accessible surface area (ASA) of bFGF residues Val88 and Phe93 and relative affinities for soluble FGFR1β-TPA fusion protein exhibited by single point bFGF muteins derived from such residues having ASA greater than 10 Å^2.

Residue	ASA (Å)	Mutant bFGF	IC_{50} (nM)	IC_{50} (mut)/ IC_{50} (wt)
Val88	62.0	V88A	5.40 ± 0.21	10.4 ± 0.81
Phe93	20.69	F93A	42 ± 10	81 ± 20

The relative affinity is the ratio of IC_{50} values calculated from the competitive binding of $_{125}$I - labeled bFGF and unlabeled wild-type bFGF on bFGF muteins, respectively, to soluble FGFR1β-TPA fusion protein (average of two experiments). N101A denotes the bFGF mutein in which the Asn residue at position 101 is replaced by Ala. The IC_{50} value for wild-type bFGF is 0.52 ± 0.04 nM.

Based on the following evidence, the loss of receptor binding affinity of F93A and V88A was not due to the effect of heparin on the FGF-FGFR interaction. First, the heparin-bFGF interaction has been shown to involve a number of residues of bFGF by site-directed mutagenesis and X-ray crystallographic approaches. Neither Val88 nor Phe93 is involved in interaction with heparin (Thompson *et al.* (1994) Biochemistry 33:3831-3840; Faham *et al.* (1996) Science 271:1116-1120). Second, the replacement of Val88 and Phe93 with alanine did not impair the ability to bind to heparin-Sepharose because these FGF muteins can be eluted from a heparin-Sepharose column with 2 M NaCl buffer. These data suggest that replacement of Val88 and Phe93 by alanine does not significantly affect heparin affinity and that there are no global conformational changes in V88A and F93A muteins.

Previous studies have shown that bFGF muteins, E96A, N104A, Y103A, and L140A exhibit greater than 200-fold reductions, suggesting

BNSDOCID <WO 9955861A2 | >

these residues are crucial for the high affinity receptor binding (Zhu *et al.* (1995) J. Biol. Chem. 270:21869-218741; Zhu *et al.* (1997) Protein Engineering 10:417-421). In contrast, replacement of either Val-88 or Phe-93 with alanine reduced the receptor binding affinity less than 100-fold, suggesting Val88 and Phe93 only contribute medium affinity binding to FGF receptor but do contribute to FGF receptor binding activity.

Site-directed mutagenesis studies on FGFR1 show that the active core of the receptor (the loop II, the inter-loop II/III sequence, the N-terminus of loop III and glycosaminoglycan) can bind to acidic fibroblast growth factor (aFGF), bFGF and KGF (Wang *et al.* (1995) J. Biol. Chem. 270:10222-10230). The high conservation of residues Tyr24, Glu96, Tyr103, Asn104, and Leu140 in these factors suggests that they bind to this active core of the receptor. Site directed mutagenesis on bFGF and modeling of the FGF receptor also indicate that the low affinity site composed of Lys110, Tyr111 and Trp114 may interact with receptor binding domains on the front of loopIII (Seddon *et al.* (1995) Biochemistry 34:731-736; Wang *et al.* (1995) J. Biol. Chem. 270:10222-10230). Hydrophobic residues, Val-88 and Phe-93 are members of a medium affinity site and about 10-15 Å from the low affinity site.

The new hydrophobic residues identified here for the FGF receptor binding elucidates the importance of the hydrophobic cluster on the surface of a molecule for the protein-protein association. Results here suggest that the surface hydrophobic patch can be used to identify regions of a protein's surface most likely to interact with its receptor. This is critical for the structure-based design of small molecule antagonists.

To explore further potentially critical hydrophobic residues near the high affinity receptor binding site on bFGF, the hydrophobic residues Phe30 and Leu138 which neighbor of Tyr24 and Tyr103 within 5 Å radius, respectively, were replaced with alanine. As shown in Table 5, both residues are solvent accessible:

Table 5. Water-accessible surface area (ASA) of bFGF residues Phe30 and Leu138 and relative affinities for soluble FGFR1β-TPA fusion protein exhibited by single point bFGF muteins derived from such residues having ASA greater than 10 Å^2.

Residue	ASA (Å)	Mutant bFGF	IC_{50} (nM)	IC_{50} (mut)/ IC_{50} (wt)
Phe30	24.10	F30A	1.38 ± 0.09	2.65 ± 0.25
Leu138	23.97	L138A	0.14 ± 0.01	0.27 ± 0.03

The relative affinity is the ratio of IC_{50} values calculated from the competitive binding of $_{125}$I - labeled bFGF and unlabeled wild-type bFGF on bFGF muteins, respectively, to soluble FGFR1β-TPA fusion protein (average of two experiments). N101A denotes the bFGF mutein in which the Asn residue at position 101 is replaced by Ala. The IC_{50} value for wild-type bFGF is 0.52 ± 0.04 nM.

Table 5 also shows that substitution of residue Phe30 by alanine gave a mutein with nearly unchanged receptor binding affinity compared with the wild-type. Replacement of residue Leu138 with an alanine residue resulted in an apparent 4.5-fold increase in the receptor binding affinity compared with the wild-type indicating that amino acid residues corresponding to this position can contribute to medium to high affinity receptor binding.

B. Mitogenic activity

The mitogenic activity of the bFGF muteins was determined by measuring the incorporation of tritiated thymidine into DNA of Rb-1 cells as described above in EXAMPLE 2C. Table 6 shows the relative mean

BNSDOCID <WO 9955861A2 I >

percent increase in cell proliferation for the bFGF mutein in which leucine 138 has been replaced by alanine compared to wild type bFGF.

TABLE 6

rh bFGF		Leu138A Mutein	
Conc. [nM]	Mean % Increase in Stimulation	Conc. [nM]	Mean % Increase in Stimulation
581	86	581	68
291	86	291	47
145	83	145	11
72.63	77	72.63	0
36.3	65	36.3	0

As illustrated in Table 6, the mitogenic activity of the Leu138A mutein was decreased by greater than 10-fold compared to wild type bFGF. The above-described data demonstrate that the leucine residue at position 138 of bFGF is critical for mitogenic activity as well as contributing to receptor binding activity (e.g., see Table 5 in EXAMPLE 3A).

CLAIMS

1. An isolated nucleic acid molecule, comprising a sequence of nucleotides that encodes a fibroblast growth factor (FGF) mutein selected from the group consisting of FGF-2, FGF-1, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9 and FGF-10; wherein,

the FGF-2 has been modified by replacement of the leucine residue at position 138 with another amino acid;

the FGF-1 has been modified by replacement of the leucine residue at position 146 with another amino acid;

the FGF-3 has been modified by replacement of the leucine residue at position 177 with another amino acid;

the FGF-4 has been modified by replacement of the histidine residue at position 201 with another amino acid;

the FGF-5 has been modified by replacement of the histidine residue at position 214 with another amino acid;

the FGF-6 has been modified by replacement of the histidine residue at position 193 with another amino acid;

the FGF-7 has been modified by replacement of the histidine residue at position 187 with another amino acid;

the FGF-8 has been modified by replacement of the lysine residue at position 176 with another amino acid;

the FGF-9 has been modified by replacement of the histidine residue at position 186 with another amino acid;

the FGF-10 has been modified by replacement of the histidine residue at position 135 with another amino acid; and

the position numbers are determined by reference to SEQ ID NOS. 1 to 10 for FGF-1 to FGF-10, respectively.

BNSDOCID <WO 9955861A2 I >

2. The nucleic acid molecule of claim 1, wherein the replacement amino acid is alanine, phenylalanine, glycine, serine, methionine, or tyrosine.

3. The nucleic acid molecule of any of claims 1 or 2 that encodes an FGF-2 mutein, wherein the sequence of nucleotides that encodes the FGF-2 mutein encodes a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2, except that the leucine residue at position 138 is replaced with alanine.

4. The nucleic acid molecule of any or claims 1-3, wherein cysteine residues that do not participate in disulfide bonding of the native moleucles are replaced with a neutral amino acid, whereby stability or aggregation is decreased and/or homogeneity of recombinantly produced proteins is increased.

5. An isolated nucleic acid molecule, comprising a sequence of nucleotides that encodes a fibroblast growth factor (FGF) mutein selected from the group consisting of FGF-2, FGF-1, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9 and FGF-10; wherein,

the FGF-2 has been modified by replacement of the valine residue at position 88 with another amino acid;

the FGF-1 has been modified by replacement of the proline residue at position 94 with another amino acid;

the FGF-3 has been modified by replacement of the tyrosine residue at position 111 with another amino acid;

the FGF-4 has been modified by replacement of the phenylalanine residue at position 151 with another amino acid;

the FGF-5 has been modified by replacement of the phenylalanine residue at position 156 with another amino acid;

the FGF-6 has been modified by replacement of the phenylalanine residue at position 143 with another amino acid;

the FGF-7 has been modified by replacement of the cysteine residue at position 133 with another amino acid;

the FGF-8 has been modified by replacement of the lysine residue at position 123 with another amino acid;

the FGF-9 has been modified by replacement of the leucine residue at position 130 with another amino acid;

the FGF-10 has been modified by replacement of the phenylalanine residue at position 79 with another amino acid; and

the position numbers are determined by reference to SEQ ID NOS. 1 to 10 for FGF-1 to FGF-10, respectively; and the replacement amino acid is selected such that the resulting mutein has substantially reduced binding affinity for FGF receptor-1 (FGFR1) compared to wild type.

6. The nucleic acid molecule of claim 5 that encodes an FGF-2 mutein, wherein the sequence of nucleotides that encodes the FGF-2 mutein encodes a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2, except that the valine residue at position 88 is replaced with alanine.

7. An isolated nucleic acid molecule, comprising a sequence of nucleotides that encodes a fibroblast growth factor (FGF) mutein selected from the group consisting of FGF-2, FGF-1, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9 and FGF-10; wherein,

the FGF-2 has been modified by replacement of the phenylalanine residue at position 93 with another amino acid;

the FGF-1 has been modified by replacement of the leucine residue at position 99 with another amino acid;

the FGF-3 has been modified by replacement of the glutamic acid residue at position 116 with another amino acid;

the FGF-4 has been modified by replacement of the threonine residue at position 156 with another amino acid;

the FGF-5 has been modified by replacement of the lysine residue at position 161 with another amino acid;

the FGF-6 has been modified by replacement of the lysine residue at position 148 with another amino acid;

the FGF-7 has been modified by replacement of the asparagine residue at position 138 with another amino acid;

the FGF-8 has been modified by replacement of the valine residue at position 128 with another amino acid;

the FGF-9 has been modified by replacement of the valine residue at position 135 with another amino acid;

the FGF-10 has been modified by replacement of the lysine residue at position 84 with another amino acid; and

the position numbers are determined by reference to SEQ ID NOS. 1 to 10 for FGF-1 to FGF-10, respectively; and the replacement amino acid is selected such that the resulting mutein has substantially reduced binding affinity for FGF receptor-1 (FGFR1) compared to wild type.

8. The nucleic acid molecule of claim 7 that encodes an FGF-2 mutein, wherein the sequence of nucleotides that encodes the FGF-2 mutein encodes a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2, except that the phenylalanine residue at position 93 is replaced with alanine.

9. The nucleic acid molecule of any of claims 1-8, wherein the replacement amino acid is alanine, glycine or serine.

10. The nucleic acid molecule of any of claims 1-9, wherein the replacement amino acid is alanine.

11. The nucleic acid molecule any of claims 1-10, wherein the FGF mutein is further modified by replacement of one or more cysteine residues with another amino acid, whereby aggregation of the resulting peptide is reduced compared to the wild type polypeptide.

12. The nucleic acid molecule of any of claims 1-11, wherein the FGF is FGF-2 and the replaced cysteine residues are Cys69 and Cys87.

13. The nucleic acid molecule of any of claims 1-12, wherein the cysteine residues are replaced with serine.

14. The nucleic acid molecule of any of claims 5-8, wherein:

the FGF mutein is further modified by replacement of the Glu positions 102, 96, 119, 159, 164, 151, 141, 131, 137 and 87 in FGF-1 - FGF-10, respectively, with an amino acid that results in an FGF mutein that does not bind to FGFR1; and

the position numbers are determined by reference to SEQ ID NO. 1-10 for FGF-1 to FGF-10, respectively.

15. The nucleic acid molecule of claim 14, wherein the replacement amino acid is alanine, phenylalanine, serine, glycine, methionine, leucine or tyrosine.

16. A fibroblast growth factor mutein polypeptide encoded by the nucleic acid molecule of any of claims 1-15.

17. A pharmaceutical composition, comprising a therapeutically effective amount of the FGF mutein encoded by the nucleic acid molecule of any of claims 1-15 in a vehicle suitable for topical, local or systemic administration, wherein the amount is effective for ameliorating at least one symptom of an FGF-mediated disorder.

18. A method of treating an FGF-mediated disorder, comprising administering a therapeutically effective amount of the pharmaceutical composition of claim 17, whereby the therapeutically effective amount of the FGF mutein ameliorates at least one symptom of the FGF-mediated disorder.

19. The method of claim 18, wherein the FGF-mediated disorder is selected from the group consisting of restenosis, in-stent restenosis,

vascular injury, ophthalmic disorders, rheumatoid arthritis and tumorigenesis.

20. A method of treating a heparin-related disorder, comprising administering a therapeutically effective amount of an FGF mutein encoded by the nucleic acid molecule of any of claims 5-8 that binds to heparin but has substantially reduced FGF receptor-1 binding activity compared to wild-type, whereby the therapeutically effective amount of the FGF mutein ameliorates at least one symptom of the heparin-related disorder.

21. The method of claim 20, wherein the heparin-related disorder is selected from the group consisting of excessive bleeding induced by heparin, ophthalmic disorders and heparin-associated thrombocytopenia and thrombosis.

22. An article of manufacture, comprising packaging material and a pharmaceutical composition of claim 17 contained within the packaging material, wherein the pharmaceutical composition is effective for antagonizing the effects of FGF, ameliorating the symptoms of an FGF-mediated disorder, or inhibiting the binding of an FGF polypeptide to an FGF receptor, and the packaging material includes a label that indicates that the pharmaceutical composition is used for antagonizing the effects of FGF, inhibiting the binding of an FGF polypeptide to an FGF receptor or treating an FGF-mediated disorder.

23. Use of an FGF mutein encoded by the nucleic acid molecule of any of claims 1-15 for the formulation of a medicament for the treatment of FGF-mediated disorders.

24. Use of an FGF mutein encoded by the nucleic acid molecule of any of claims 1-15 for the treament of FGF-mediated disorders.

25. Use of an FGF mutein encoded by the nucleic acid molecule of any of claims 5-8 that binds to heparin but has substantially reduced

FGF receptor-1 binding activity compared to wild-type for the formulation of a medicament for the treatment of heparin-related disorders.

26. Use of an FGF mutein encoded by the nucleic acid molecule of any of claims 5-8 that binds to heparin but has substantially reduced FGF receptor-1 binding activity compared to wild-type for the treament of FGF-mediated disorders.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT:
(A) NAME: Eisai, Ltd.
(B) STREET: 112-88 4-6-10 Koishikawa
(C) CITY: Bunkyo-ku Tokyo
(D) STATE:
(E) COUNTRY: Japan
(F) POSTAL CODE (ZIP):

(i) INVENTOR:
(A) NAME: Hengyi Zhu
(B) STREET: 4941 Brookburn Drive
(C) CITY: San Diego
(D) STATE: California
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 92130

(i) INVENTOR:
(A) NAME: Kalyanaraman Ramnarayan
(B) STREET: 11674 Springside Rd.
(C) CITY: San Diego
(D) STATE: California
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 92128

(ii) TITLE OF INVENTION: FIBROBLAST GROWTH FACTOR MUTEIN COMPOSITIONS AND METHODS OF USE THEREFOR

(iii) NUMBER OF SEQUENCES: 13

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Heller Ehrman White & McAuliffe
(B) STREET: 4250 Executive Square, 7th Floor
(C) CITY: La Jolla
(D) STATE: California
(E) COUNTRY: US
(F) ZIP: 92037

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 1.5

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: herewith
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 09/067,929
(B) FILING DATE: 28-APR-98

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Seidman, Stephanie L.

(B) REGISTRATION NUMBER: 33,779
(C) REFERENCE/DOCKET NUMBER: 24732-1209PC

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (619) 450-8400
(B) TELEFAX: (619) 587-5360

2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 155 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Met Ala Glu Gly Glu Ile Thr Thr Phe Thr Ala Leu Thr Glu Lys Phe
1               5                  10                  15

Asn Leu Pro Pro Gly Asn Tyr Lys Lys Pro Lys Leu Leu Tyr Cys Ser
            20                  25                  30

Asn Gly Gly His Phe Leu Arg Ile Leu Pro Asp Gly Thr Val Asp Gly
        35                  40                  45

Thr Arg Asp Arg Ser Asp Gln His Ile Gln Leu Gln Leu Ser Ala Glu
    50                  55                  60

Ser Val Gly Glu Val Tyr Ile Lys Ser Thr Glu Thr Gly Gln Tyr Leu
65                  70                  75                  80

Ala Met Asp Thr Asp Gly Leu Leu Tyr Gly Ser Gln Thr Pro Asn Glu
                85                  90                  95

Glu Cys Leu Phe Leu Glu Arg Leu Glu Glu Asn His Tyr Asn Thr Tyr
            100                 105                 110

Ile Ser Lys Lys His Ala Glu Lys Asn Trp Phe Val Gly Leu Lys Lys
        115                 120                 125

Asn Gly Ser Cys Lys Arg Gly Pro Arg Thr His Tyr Gly Gln Lys Ala
    130                 135                 140

Ile Leu Phe Leu Pro Leu Pro Val Ser Ser Asp
145                 150                 155
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 468 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS

BNSDOCID <WO 9955861A2 I >

(B) LOCATION: 1..468

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 1..468
(D) OTHER INFORMATION: /product= "bFGF"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
ATG GCA GCA GGA TCA ATA ACA ACA TTA CCC GCC TTG CCC GAG GAT GGC          48
Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly
-9              -5                   1                   5

GGC AGC GGC GCC TTC CCG CCC GGC CAC TTC AAG GAC CCC AAG CGG CTG          96
Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu
      10                  15                  20

TAC TGC AAA AAC GGG GGC TTC TTC CTG CGC ATC CAC CCC GAC GGC CGA         144
Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His Pro Asp Gly Arg
    25                  30                  35

GTT GAC GGG GTC CGG GAG AAG AGC GAC CCT CAC ATC AAG CTT CAA CTT         192
Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu
40                  45                  50                  55

CAG GCA GAA GAG AGA GGA GTT GTG TCT ATC AAA GGA GTG TGT GCT AAC         240
Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn
                60                  65                  70

CGT TAC CTG GCT ATG AAG GAA GAT GGA AGA TTA CTG GCT TCT AAA TGT         288
Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys
            75                  80                  85

GTT ACG GAT GAG TGT TTC TTT TTT GAA CGA TTG GAA TCT AAT AAC TAC         336
Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr
        90                  95                  100

AAT ACT TAC CGG TCA AGG AAA TAC ACC AGT TGG TAT GTG GCA TTG AAA         384
Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys
    105                 110                 115

CGA ACT GGG CAG TAT AAA CTT GGA TCC AAA ACA GGA CCT GGG CAG AAA         432
Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys
120                 125                 130                 135

GCT ATA CTT TTT CTT CCA ATG TCT GCT AAG AGC TGA                         468
Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser  *
                140                 145
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 239 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Gly Leu Ile Trp Leu Leu Leu Leu Ser Leu Leu Glu Pro Gly Trp
1               5                  10                  15

Pro Ala Ala Gly Pro Gly Ala Arg Leu Arg Arg Asp Ala Gly Gly Arg
            20                  25                  30

Gly Gly Val Tyr Glu His Leu Gly Gly Ala Pro Arg Arg Arg Lys Leu
        35                  40                  45

Tyr Cys Ala Thr Lys Tyr His Leu Gln Leu His Pro Ser Gly Arg Val
    50                  55                  60

Asn Gly Ser Leu Glu Asn Ser Ala Tyr Ser Ile Leu Glu Ile Thr Ala
65                  70                  75                  80

Val Glu Val Gly Ile Val Ala Ile Arg Gly Leu Phe Ser Gly Arg Tyr
                85                  90                  95

Leu Ala Met Asn Lys Arg Gly Arg Leu Tyr Ala Ser Glu His Tyr Ser
            100                 105                 110

Ala Glu Cys Glu Phe Val Glu Arg Ile His Glu Leu Gly Tyr Asn Thr
        115                 120                 125

Tyr Ala Ser Arg Leu Tyr Arg Thr Val Ser Ser Thr Pro Gly Ala Arg
    130                 135                 140

Arg Gln Pro Ser Ala Glu Arg Leu Trp Tyr Val Ser Val Asn Gly Lys
145                 150                 155                 160

Gly Arg Pro Arg Arg Gly Phe Lys Thr Arg Arg Thr Gln Lys Ser Ser
                165                 170                 175

Leu Phe Leu Pro Arg Val Leu Asp His Arg Asp His Glu Met Val Arg
            180                 185                 190

Gln Leu Gln Ser Gly Leu Pro Arg Pro Pro Gly Lys Gly Val Gln Pro
        195                 200                 205

Arg Arg Arg Arg Gln Lys Gln Ser Pro Asp Asn Leu Glu Pro Ser His
    210                 215                 220

Val Gln Ala Ser Arg Leu Gly Ser Gln Leu Glu Ala Ser Ala His
225                 230                 235
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 206 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ser Gly Pro Gly Thr Ala Ala Val Ala Leu Leu Pro Ala Val Leu
1               5                  10                  15
```


BNSDOCID <WO 9955861A2 I >

```
Leu Ala Leu Leu Ala Pro Trp Ala Gly Arg Gly Gly Ala Ala Ala Pro
            20                  25                  30

Thr Ala Pro Asn Gly Thr Leu Glu Ala Glu Leu Glu Arg Arg Trp Glu
        35                  40                  45

Ser Leu Val Ala Leu Ser Leu Ala Arg Leu Pro Val Ala Ala Gln Pro
    50                  55                  60

Lys Glu Ala Ala Val Gln Ser Gly Ala Gly Asp Tyr Leu Leu Gly Ile
65                  70                  75                  80

Lys Arg Leu Arg Arg Leu Tyr Cys Asn Val Gly Ile Gly Phe His Leu
                85                  90                  95

Gln Ala Leu Pro Asp Gly Arg Ile Gly Gly Ala His Ala Asp Thr Arg
            100                 105                 110

Asp Ser Leu Leu Glu Leu Ser Pro Val Glu Arg Gly Val Val Ser Ile
        115                 120                 125

Phe Gly Val Ala Ser Arg Phe Phe Val Ala Met Ser Ser Lys Gly Lys
    130                 135                 140

Leu Tyr Gly Ser Pro Phe Phe Thr Asp Glu Cys Thr Phe Lys Glu Ile
145                 150                 155                 160

Leu Leu Pro Asn Asn Tyr Asn Ala Tyr Glu Ser Tyr Lys Tyr Pro Gly
                165                 170                 175

Met Phe Ile Ala Leu Ser Lys Asn Gly Lys Thr Lys Lys Gly Asn Arg
            180                 185                 190

Val Ser Pro Thr Met Lys Val Thr His Phe Leu Pro Arg Leu
        195                 200                 205
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 268 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Ser Leu Ser Phe Leu Leu Leu Leu Phe Phe Ser His Leu Ile Leu
1               5                   10                  15

Ser Ala Trp Ala His Gly Glu Lys Arg Leu Ala Pro Lys Gly Gln Pro
            20                  25                  30

Gly Pro Ala Ala Thr Asp Arg Asn Pro Ile Gly Ser Ser Ser Arg Gln
        35                  40                  45

Ser Ser Ser Ser Ala Met Ser Ser Ser Ser Ala Ser Ser Ser Pro Ala
    50                  55                  60
```

Ala Ser Leu Gly Ser Gln Gly Ser Gly Leu Glu Gln Ser Ser Phe Gln
65 70 75 80

Trp Ser Pro Ser Gly Arg Arg Thr Gly Ser Leu Tyr Cys Arg Val Gly
85 90 95

Ile Gly Phe His Leu Gln Ile Tyr Pro Asp Gly Lys Val Asn Gly Ser
100 105 110

His Glu Ala Asn Met Leu Ser Val Leu Glu Ile Phe Ala Val Ser Gln
115 120 125

Gly Ile Val Gly Ile Arg Gly Val Phe Ser Asn Lys Phe Leu Ala Met
130 135 140

Ser Lys Lys Gly Lys Leu His Ala Ser Ala Lys Phe Thr Asp Asp Cys
145 150 155 160

Lys Phe Arg Glu Arg Phe Gln Glu Asn Ser Tyr Asn Thr Tyr Ala Ser
165 170 175

Ala Ile His Arg Thr Glu Lys Thr Gly Arg Glu Trp Tyr Val Ala Leu
180 185 190

Asn Lys Arg Gly Lys Ala Lys Arg Gly Cys Ser Pro Arg Val Lys Pro
195 200 205

Gln His Ile Ser Thr His Phe Leu Pro Arg Phe Lys Gln Ser Glu Gln
210 215 220

Pro Glu Leu Ser Phe Thr Val Thr Val Pro Glu Lys Lys Asn Pro Pro
225 230 235 240

Ser Pro Ile Lys Ser Lys Ile Pro Leu Ser Ala Pro Arg Lys Asn Thr
245 250 255

Asn Ser Val Lys Tyr Arg Leu Lys Phe Arg Phe Gly
260 265

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 198 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ser Arg Gly Ala Gly Arg Leu Gln Gly Thr Leu Trp Ala Leu Val
1 5 10 15

Phe Leu Gly Ile Leu Val Gly Met Val Val Pro Ser Pro Ala Gly Thr
20 25 30

Arg Ala Asn Asn Thr Leu Leu Asp Ser Arg Gly Trp Gly Thr Leu Leu
35 40 45

BNSDOCID <WO 9955861A2 I >

Ser Arg Ser Arg Ala Gly Leu Ala Gly Glu Ile Ala Gly Val Asn Trp
50 55 60

Glu Ser Gly Tyr Leu Val Gly Ile Lys Arg Gln Arg Arg Leu Tyr Cys
65 70 75 80

Asn Val Gly Ile Gly Phe His Leu Gln Val Leu Pro Asp Gly Arg Ile
85 90 95

Ser Gly Thr His Glu Glu Asn Pro Tyr Ser Leu Leu Glu Ile Ser Thr
100 105 110

Val Glu Arg Gly Val Val Ser Leu Phe Gly Val Arg Ser Ala Leu Phe
115 120 125

Val Ala Met Asn Ser Lys Gly Arg Leu Tyr Ala Thr Pro Ser Phe Gln
130 135 140

Glu Glu Cys Lys Phe Arg Glu Thr Leu Leu Pro Asn Asn Tyr Asn Ala
145 150 155 160

Tyr Glu Ser Asp Leu Tyr Gln Gly Thr Tyr Ile Ala Leu Ser Lys Tyr
165 170 175

Gly Arg Val Lys Arg Gly Ser Lys Val Ser Pro Ile Met Thr Val Thr
180 185 190

His Phe Leu Pro Arg Ile
195

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 194 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met His Lys Trp Ile Leu Thr Trp Ile Leu Pro Thr Leu Leu Tyr Arg
1 5 10 15

Ser Cys Phe His Ile Ile Cys Leu Val Gly Thr Ile Ser Leu Ala Cys
20 25 30

Asn Asp Met Thr Pro Glu Gln Met Ala Thr Asn Val Asn Cys Ser Ser
35 40 45

Pro Glu Arg His Thr Arg Ser Tyr Asp Tyr Met Glu Gly Gly Asp Ile
50 55 60

Arg Val Arg Arg Leu Phe Cys Arg Thr Gln Trp Tyr Leu Arg Ile Asp
65 70 75 80

Lys Arg Gly Lys Val Lys Gly Thr Gln Glu Met Lys Asn Asn Tyr Asn
85 90 95

```
Ile Met Glu Ile Arg Thr Val Ala Val Gly Ile Val Ala Ile Lys Gly
            100                 105                 110

Val Glu Ser Glu Phe Tyr Leu Ala Met Asn Lys Glu Gly Lys Leu Tyr
        115                 120                 125

Ala Lys Lys Glu Cys Asn Glu Asp Cys Asn Phe Lys Glu Leu Ile Leu
    130                 135                 140

Glu Asn His Tyr Asn Thr Tyr Ala Ser Ala Lys Trp Thr His Asn Gly
145                 150                 155                 160

Gly Glu Met Phe Val Ala Leu Asn Gln Lys Gly Ile Pro Val Arg Gly
                165                 170                 175

Lys Lys Thr Lys Lys Glu Gln Lys Thr Ala His Phe Leu Pro Met Ala
            180                 185                 190

Ile Thr
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 215 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Gly Ser Pro Arg Ser Ala Leu Ser Cys Leu Leu Leu His Leu Leu
1               5                   10                  15

Val Leu Cys Leu Gln Ala Gln Val Thr Val Gln Ser Ser Pro Asn Phe
            20                  25                  30

Thr Gln His Val Arg Glu Gln Ser Leu Val Thr Asp Gln Leu Ser Arg
        35                  40                  45

Arg Leu Ile Arg Thr Tyr Gln Leu Tyr Ser Arg Thr Ser Gly Lys His
    50                  55                  60

Val Gln Val Leu Ala Asn Lys Arg Ile Asn Ala Met Ala Glu Asp Gly
65                  70                  75                  80

Asp Pro Phe Ala Lys Leu Ile Val Glu Thr Asp Thr Phe Gly Ser Arg
                85                  90                  95

Val Arg Val Arg Gly Ala Glu Thr Gly Leu Tyr Ile Cys Met Asn Lys
            100                 105                 110

Lys Gly Lys Leu Ile Ala Lys Ser Asn Gly Lys Gly Lys Asp Cys Val
        115                 120                 125

Phe Thr Glu Ile Val Leu Glu Asn Asn Tyr Asn Ala Leu Gln Asn Ala
    130                 135                 140

Lys Tyr Glu Gly Trp Tyr Met Ala Phe Thr Arg Lys Gly Arg Pro Arg
145                 150                 155                 160
```


BNSDOCID <WO 9955861A2 >

Lys Gly Ser Lys Thr Arg Gln His Gln Arg Glu Val His Phe Met Lys
165 170 175

Arg Leu Pro Arg Gly His His Thr Thr Glu Gln Ser Leu Arg Phe Glu
180 185 190

Phe Leu Asn Tyr Pro Pro Phe Thr Arg Ser Leu Arg Gly Ser Gln Arg
195 200 205

Thr Trp Ala Pro Glu Pro Arg
210 215

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 208 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Ala Pro Leu Gly Glu Val Gly Asn Tyr Phe Gly Val Gln Asp Ala
1 5 10 15

Val Pro Phe Gly Asn Val Pro Val Leu Pro Val Asp Ser Pro Val Leu
20 25 30

Leu Ser Asp His Leu Gly Gln Ser Glu Ala Gly Gly Leu Pro Arg Gly
35 40 45

Pro Ala Val Thr Asp Leu Asp His Leu Lys Gly Ile Leu Arg Arg Arg
50 55 60

Gln Leu Tyr Cys Arg Thr Gly Phe His Leu Glu Ile Phe Pro Asn Gly
65 70 75 80

Thr Ile Gln Gly Thr Arg Lys Asp His Ser Arg Phe Gly Ile Leu Glu
85 90 95

Phe Ile Ser Ile Ala Val Gly Leu Val Ser Ile Arg Gly Val Asp Ser
100 105 110

Gly Leu Tyr Leu Gly Met Asn Glu Lys Gly Glu Leu Tyr Gly Ser Glu
115 120 125

Lys Leu Thr Gln Glu Cys Val Phe Arg Glu Gln Phe Glu Glu Asn Trp
130 135 140

Tyr Asn Thr Tyr Ser Ser Asn Leu Tyr Lys His Val Asp Thr Gly Arg
145 150 155 160

Arg Tyr Tyr Val Ala Leu Asn Lys Asp Gly Thr Pro Arg Glu Gly Thr
165 170 175

Arg Thr Lys Arg His Gln Lys Phe Thr His Phe Leu Pro Arg Pro Val
180 185 190

Asp Pro Asp Lys Val Pro Glu Leu Tyr Lys Asp Ile Leu Ser Gln Ser
195 200 205

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 181 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met Glu Ser Lys Glu Pro Gln Leu Lys Gly Ile Val Thr Arg Leu Phe
 1               5                  10                  15
Ser Gln Gln Gly Tyr Phe Leu Gln Met His Pro Asp Gly Thr Ile Asp
            20                  25                  30
Gly Thr Lys Asp Glu Asn Ser Asp Tyr Thr Leu Phe Asn Leu Ile Pro
        35                  40                  45
Val Gly Leu Arg Val Val Ala Ile Gln Gly Val Lys Ala Ser Leu Tyr
    50                  55                  60
Val Ala Met Asn Gly Glu Gly Tyr Leu Tyr Ser Ser Asp Val Phe Thr
65                  70                  75                  80
Pro Glu Cys Lys Phe Lys Glu Ser Val Phe Glu Asn Tyr Tyr Val Ile
                85                  90                  95
Tyr Ser Ser Thr Leu Tyr Arg Gln Gln Glu Ser Gly Arg Ala Trp Phe
            100                 105                 110
Leu Gly Leu Asn Lys Glu Gly Gln Ile Met Lys Gly Asn Arg Val Lys
        115                 120                 125
Lys Thr Lys Pro Ser Ser His Phe Val Pro Lys Pro Ile Glu Val Cys
    130                 135                 140
Met Tyr Arg Glu Pro Ser Leu His Glu Ile Gly Glu Lys Gln Gly Arg
145                 150                 155                 160
Ser Arg Lys Ser Ser Gly Thr Pro Thr Met Asn Gly Gly Lys Val Val
                165                 170                 175
Asn Gln Asp Ser Thr
            180
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1440 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(ix) FEATURE:

(A) NAME/KEY: Coding Sequence
(B) LOCATION: 9...1427
(D) OTHER INFORMATION: FGFR1-tPA fusion protein

BNSDOCID <WO 9955861A2 I >

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
AAGCTTGG ATG TGG AGC TGG AAG TGC CTC CTC TTC TGG GCT GTG CTG GTC      50
         Met Trp Ser Trp Lys Cys Leu Leu Phe Trp Ala Val Leu Val
          1               5                  10

ACA GCA ACA CTC TGC ACC GCT AGG CCG TCC CCG ACC TTG CCT GAA CAA      98
Thr Ala Thr Leu Cys Thr Ala Arg Pro Ser Pro Thr Leu Pro Glu Gln
15                  20                  25                  30

GAT GCT CTC CCC TCC TCG GAG GAT GAT GAT GAT GAT GAT GAC TCC TCT     146
Asp Ala Leu Pro Ser Ser Glu Asp Asp Asp Asp Asp Asp Asp Ser Ser
                35                  40                  45

TCA GAG GAG AAA GAA ACA GAT AAC ACC AAA CCA AAC CCC GTA GCT CCA     194
Ser Glu Glu Lys Glu Thr Asp Asn Thr Lys Pro Asn Pro Val Ala Pro
            50                  55                  60

TAT TGG ACA TCC CCA GAA AAG ATG GAA AAG AAA TTG CAT GCA GTG CCG     242
Tyr Trp Thr Ser Pro Glu Lys Met Glu Lys Lys Leu His Ala Val Pro
        65                  70                  75

GCT GCC AAG ACA GTG AAG TTC AAA TGC CCT TCC AGT GGG ACC CCA AAC     290
Ala Ala Lys Thr Val Lys Phe Lys Cys Pro Ser Ser Gly Thr Pro Asn
    80                  85                  90

CCC ACA CTG CGC TGG TTG AAA AAT GGC AAA GAA TTC AAA CCT GAC CAC     338
Pro Thr Leu Arg Trp Leu Lys Asn Gly Lys Glu Phe Lys Pro Asp His
95                  100                 105                 110

AGA ATT GGA GGC TAC AAG GTC CGT TAT GCC ACC TGG AGC ATC ATA ATG     386
Arg Ile Gly Gly Tyr Lys Val Arg Tyr Ala Thr Trp Ser Ile Ile Met
                115                 120                 125

GAC TCT GTG GTG CCC TCT GAC AAG GGC AAC TAC ACC TGC ATT GTG GAG     434
Asp Ser Val Val Pro Ser Asp Lys Gly Asn Tyr Thr Cys Ile Val Glu
            130                 135                 140

AAT GAG TAC GGC AGC ATC AAC CAC ACA TAC CAG CTG GAT GTC GTG GAG     482
Asn Glu Tyr Gly Ser Ile Asn His Thr Tyr Gln Leu Asp Val Val Glu
        145                 150                 155

CGG TCC CCT CAC CGG CCC ATC CTG CAA GCA GGG TTG CCC GCC AAC AAA     530
Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn Lys
    160                 165                 170

ACA GTG GCC CTG GGT AGC AAC GTG GAG TTC ATG TGT AAG GTG TAC AGT     578
Thr Val Ala Leu Gly Ser Asn Val Glu Phe Met Cys Lys Val Tyr Ser
175                 180                 185                 190

GAC CCG CAG CCG CAC ATC CAG TGG CTA AAG CAC ATC GAG GTG AAT GGG     626
Asp Pro Gln Pro His Ile Gln Trp Leu Lys His Ile Glu Val Asn Gly
                195                 200                 205

AGC AAG ATT GGC CCA GAC AAC CTG CCT TAT GTC CAG ATC TTG AAG ACT     674
Ser Lys Ile Gly Pro Asp Asn Leu Pro Tyr Val Gln Ile Leu Lys Thr
            210                 215                 220

GCT GGA GTT AAT ACC ACC GAC AAA GAG ATG GAC GTG CTT CAC TTA AGA     722
```

```
Ala Gly Val Asn Thr Thr Asp Lys Glu Met Asp Val Leu His Leu Arg
        225                 230                 235

AAT GTC TCC TTT GAG GAC GCA GGG GAG TAT ACG TGC TTG GCG GGT AAC      770
Asn Val Ser Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly Asn
    240                 245                 250

TCT ATC GGA CTC TCC CAT CAC TCT GCA TGG TTG ACC GTT CTG GAA GCC      818
Ser Ile Gly Leu Ser His His Ser Ala Trp Leu Thr Val Leu Glu Ala
255                 260                 265                 270

CTG GAA GAG AGG CCG GCA GTG ATG ACC TCG CCC CTG TAC GTC GAC GCC      866
Leu Glu Glu Arg Pro Ala Val Met Thr Ser Pro Leu Tyr Val Asp Ala
                275                 280                 285

CGA TTC CCA AGA GGA GCC AGA TCT TAC CAA GTG ATC TGC AGA GAT GAA      914
Arg Phe Pro Arg Gly Ala Arg Ser Tyr Gln Val Ile Cys Arg Asp Glu
            290                 295                 300

AAA ACG CAG ATG ATA TAC CAG CAA CAT CAG TCA TGG CTG CGC CCT GTG      962
Lys Thr Gln Met Ile Tyr Gln Gln His Gln Ser Trp Leu Arg Pro Val
        305                 310                 315

CTC AGA AGC AAC CGG GTG GAA TAT TGC TGG TGC AAC AGT GGC AGG GCA     1010
Leu Arg Ser Asn Arg Val Glu Tyr Cys Trp Cys Asn Ser Gly Arg Ala
    320                 325                 330

CAG TGC CAC TCA GTG CCT GTC AAA AGT TGC AGC GAG CCA AGG TGT TTC     1058
Gln Cys His Ser Val Pro Val Lys Ser Cys Ser Glu Pro Arg Cys Phe
335                 340                 345                 350

AAC GGG GGC ACC TGC CAG CAG GCC CTG TAC TTC TCA GAT TTC GTG TGC     1106
Asn Gly Gly Thr Cys Gln Gln Ala Leu Tyr Phe Ser Asp Phe Val Cys
                355                 360                 365

CAG TGC CCC GAA GGA TTT GCT GGG AAG TGC TGT GAA ATA GAT ACC AGG     1154
Gln Cys Pro Glu Gly Phe Ala Gly Lys Cys Cys Glu Ile Asp Thr Arg
            370                 375                 380

GCC ACG TGC TAC GAG GAC CAG GGC ATC AGC TAC AGG GGC ACG TGG AGC     1202
Ala Thr Cys Tyr Glu Asp Gln Gly Ile Ser Tyr Arg Gly Thr Trp Ser
        385                 390                 395

ACA GCG GAG AGT GGC GCC GAG TGC ACC AAC TGG AAC AGC AGC GCG TTG     1250
Thr Ala Glu Ser Gly Ala Glu Cys Thr Asn Trp Asn Ser Ser Ala Leu
    400                 405                 410

GCC CAG AAG CCC TAC AGC GGG CGG AGG CCA GAC GCC ATC AGG CTG GGC     1298
Ala Gln Lys Pro Tyr Ser Gly Arg Arg Pro Asp Ala Ile Arg Leu Gly
415                 420                 425                 430

CTG GGG AAC CAC AAC TAC TGC AGA AAC CCA GAT CGA GAC TCA AAG CCC     1346
Leu Gly Asn His Asn Tyr Cys Arg Asn Pro Asp Arg Asp Ser Lys Pro
                435                 440                 445

TGG TGC TAC GTC TTT AAG GCG GGG AAG TAC AGC TCA GAG TTC TGC AGC     1394
Trp Cys Tyr Val Phe Lys Ala Gly Lys Tyr Ser Ser Glu Phe Cys Ser
            450                 455                 460

ACC CCT GCC TGC TCT GAG GGA AAC AGT GAC TGA TACTTTGGGA TCC          1440
Thr Pro Ala Cys Ser Glu Gly Asn Ser Asp  *
```


BNSDOCID <WO 9955861A2 I >

465 470

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 472 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Met Trp Ser Trp Lys Cys Leu Leu Phe Trp Ala Val Leu Val Thr Ala
 1               5                  10                  15
Thr Leu Cys Thr Ala Arg Pro Ser Pro Thr Leu Pro Glu Gln Asp Ala
            20                  25                  30
Leu Pro Ser Ser Glu Asp Asp Asp Asp Asp Asp Asp Ser Ser Ser Glu
        35                  40                  45
Glu Lys Glu Thr Asp Asn Thr Lys Pro Asn Pro Val Ala Pro Tyr Trp
    50                  55                  60
Thr Ser Pro Glu Lys Met Glu Lys Lys Leu His Ala Val Pro Ala Ala
65                  70                  75                  80
Lys Thr Val Lys Phe Lys Cys Pro Ser Ser Gly Thr Pro Asn Pro Thr
                85                  90                  95
Leu Arg Trp Leu Lys Asn Gly Lys Glu Phe Lys Pro Asp His Arg Ile
            100                 105                 110
Gly Gly Tyr Lys Val Arg Tyr Ala Thr Trp Ser Ile Ile Met Asp Ser
        115                 120                 125
Val Val Pro Ser Asp Lys Gly Asn Tyr Thr Cys Ile Val Glu Asn Glu
    130                 135                 140
Tyr Gly Ser Ile Asn His Thr Tyr Gln Leu Asp Val Val Glu Arg Ser
145                 150                 155                 160
Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn Lys Thr Val
                165                 170                 175
Ala Leu Gly Ser Asn Val Glu Phe Met Cys Lys Val Tyr Ser Asp Pro
            180                 185                 190
Gln Pro His Ile Gln Trp Leu Lys His Ile Glu Val Asn Gly Ser Lys
        195                 200                 205
Ile Gly Pro Asp Asn Leu Pro Tyr Val Gln Ile Leu Lys Thr Ala Gly
    210                 215                 220
Val Asn Thr Thr Asp Lys Glu Met Asp Val Leu His Leu Arg Asn Val
225                 230                 235                 240
Ser Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly Asn Ser Ile
                245                 250                 255
Gly Leu Ser His His Ser Ala Trp Leu Thr Val Leu Glu Ala Leu Glu
            260                 265                 270
Glu Arg Pro Ala Val Met Thr Ser Pro Leu Tyr Val Asp Ala Arg Phe
        275                 280                 285
Pro Arg Gly Ala Arg Ser Tyr Gln Val Ile Cys Arg Asp Glu Lys Thr
    290                 295                 300
Gln Met Ile Tyr Gln Gln His Gln Ser Trp Leu Arg Pro Val Leu Arg
305                 310                 315                 320
Ser Asn Arg Val Glu Tyr Cys Trp Cys Asn Ser Gly Arg Ala Gln Cys
                325                 330                 335
```

```
His Ser Val Pro Val Lys Ser Cys Ser Glu Pro Arg Cys Phe Asn Gly
            340                 345                 350
Gly Thr Cys Gln Gln Ala Leu Tyr Phe Ser Asp Phe Val Cys Gln Cys
        355                 360                 365
Pro Glu Gly Phe Ala Gly Lys Cys Cys Glu Ile Asp Thr Arg Ala Thr
    370                 375                 380
Cys Tyr Glu Asp Gln Gly Ile Ser Tyr Arg Gly Thr Trp Ser Thr Ala
385                 390                 395                 400
Glu Ser Gly Ala Glu Cys Thr Asn Trp Asn Ser Ser Ala Leu Ala Gln
                405                 410                 415
Lys Pro Tyr Ser Gly Arg Arg Pro Asp Ala Ile Arg Leu Gly Leu Gly
            420                 425                 430
Asn His Asn Tyr Cys Arg Asn Pro Asp Arg Asp Ser Lys Pro Trp Cys
        435                 440                 445
Tyr Val Phe Lys Ala Gly Lys Tyr Ser Ser Glu Phe Cys Ser Thr Pro
    450                 455                 460
Ala Cys Ser Glu Gly Asn Ser Asp
465                 470
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 468 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..468

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 1..468
(D) OTHER INFORMATION: /product= synthetic "bFGF"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
ATG GCA GCC GGG AGC ATC ACC ACG CTG CCC GCC CTT CCG GAG GAT GGC        48
Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly
-9              -5                  1               5

GGC AGC GGC GCC TTC CCG CCC GGG CAC TTC AAG GAC CCC AAG CGG CTG        96
Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu
        10                  15                  20

TAC TGC AAA AAC GGG GGC TTC TTC CTG CGC ATC CAC CCC GAC GGC CGA       144
Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His Pro Asp Gly Arg
    25                  30                  35

GTT GAC GGG GTC CGG GAG AAG AGC GAC CCT CAC ATC AAG CTA CAA CTT       192
Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu
40                  45                  50                  55

CAA GCA GAA GAG AGA GGA GTT GTG TCT ATC AAA GGA GTG TGT GCT AAC       240
Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn
                60                  65                  70
```


BNSDOCID <WO 9955861A2 I >

```
CGG TAC CTG GCT ATG AAG GAA GAT GGA AGA TTA CTG GCT TCT AAA TGT          288
Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys
            75                  80                  85

GTT ACG GAT GAG TGT TTC TTT TTT GAA CGA TTG GAA TCT AAT AAC TAC          336
Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr
        90                  95                  100

AAT ACT TAC CGG TCT AGA AAA TAC ACC AGT TGG TAT GTG GCA TTG AAA          384
Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys
    105                 110                 115

CGA ACT GGG CAG TAT AAA CTT GGT TCC AAA ACA GGA CCT GGG CAG AAA          432
Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys
120                 125                 130                 135

GCT ATA CTT TTT CTT CCA ATG TCT GCT AAG AGC TGA                          468
Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser  *
                140                 145
```

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT:
(A) NAME: Eisai, Ltd.
(B) STREET: 112-88 4-6-10 Koishikawa
(C) CITY: Bunkyo-ku Tokyo
(D) STATE:
(E) COUNTRY: Japan
(F) POSTAL CODE (ZIP):

(i) INVENTOR:
(A) NAME: Hengyi Zhu
(B) STREET: 4941 Brookburn Drive
(C) CITY: San Diego
(D) STATE: California
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 92130

(i) INVENTOR:
(A) NAME: Kalyanaraman Ramnarayan
(B) STREET: 11674 Springside Rd.
(C) CITY: San Diego
(D) STATE: California
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 92128

(ii) TITLE OF INVENTION: FIBROBLAST GROWTH FACTOR MUTEIN COMPOSITIONS AND METHODS OF USE THEREFOR

(iii) NUMBER OF SEQUENCES: 13

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Heller Ehrman White & McAuliffe
(B) STREET: 4250 Executive Square, 7th Floor
(C) CITY: La Jolla
(D) STATE: California
(E) COUNTRY: US
(F) ZIP: 92037

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 1.5

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: herewith
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 09/067,929
(B) FILING DATE: 28-APR-98

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Seidman, Stephanie L.

(B) REGISTRATION NUMBER: 33,779
(C) REFERENCE/DOCKET NUMBER: 24732-1209PC

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (619) 450-8400
(B) TELEFAX: (619) 587-5360

2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 155 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Ala Glu Gly Glu Ile Thr Thr Phe Thr Ala Leu Thr Glu Lys Phe
1 5 10 15

Asn Leu Pro Pro Gly Asn Tyr Lys Lys Pro Lys Leu Leu Tyr Cys Ser
20 25 30

Asn Gly Gly His Phe Leu Arg Ile Leu Pro Asp Gly Thr Val Asp Gly
35 40 45

Thr Arg Asp Arg Ser Asp Gln His Ile Gln Leu Gln Leu Ser Ala Glu
50 55 60

Ser Val Gly Glu Val Tyr Ile Lys Ser Thr Glu Thr Gly Gln Tyr Leu
65 70 75 80

Ala Met Asp Thr Asp Gly Leu Leu Tyr Gly Ser Gln Thr Pro Asn Glu
85 90 95

Glu Cys Leu Phe Leu Glu Arg Leu Glu Glu Asn His Tyr Asn Thr Tyr
100 105 110

Ile Ser Lys Lys His Ala Glu Lys Asn Trp Phe Val Gly Leu Lys Lys
115 120 125

Asn Gly Ser Cys Lys Arg Gly Pro Arg Thr His Tyr Gly Gln Lys Ala
130 135 140

Ile Leu Phe Leu Pro Leu Pro Val Ser Ser Asp
145 150 155

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 468 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS

BNSDOCID <WO 9955861A2T1 >

(B) LOCATION: 1..468

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 1..468
(D) OTHER INFORMATION: /product= "bFGF"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
ATG GCA GCA GGA TCA ATA ACA ACA TTA CCC GCC TTG CCC GAG GAT GGC      48
Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly
-9                  -5                  1                   5

GGC AGC GGC GCC TTC CCG CCC GGC CAC TTC AAG GAC CCC AAG CGG CTG      96
Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu
          10                  15                  20

TAC TGC AAA AAC GGG GGC TTC TTC CTG CGC ATC CAC CCC GAC GGC CGA     144
Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His Pro Asp Gly Arg
      25                  30                  35

GTT GAC GGG GTC CGG GAG AAG AGC GAC CCT CAC ATC AAG CTT CAA CTT     192
Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu
  40                  45                  50                  55

CAA GCA GAA GAG AGA GGA GTT GTG TCT ATC AAA GGA GTG TGT GCT AAC     240
Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn
                  60                  65                  70

CGT TAC CTG GCT ATG AAG GAA GAT GGA AGA TTA CTG GCT TCT AAA TGT     288
Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys
              75                  80                  85

GTT ACG GAT GAG TGT TTC TTT TTT GAA CGA TTG GAA TCT AAT AAC TAC     336
Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr
          90                  95                  100

AAT ACT TAC CGG TCA AGG AAA TAC ACC AGT TGG TAT GTG GCA TTG AAA     384
Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys
      105                 110                 115

CGA ACT GGG CAG TAT AAA CTT GGA TCC AAA ACA GGA CCT GGG CAG AAA     432
Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys
  120                 125                 130                 135

GCT ATA CTT TTT CTT CCA ATG TCT GCT AAG AGC TGA                     468
Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser  *
                  140                 145
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 239 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Gly Leu Ile Trp Leu Leu Leu Leu Ser Leu Leu Glu Pro Gly Trp
1               5                  10                  15

Pro Ala Ala Gly Pro Gly Ala Arg Leu Arg Arg Asp Ala Gly Gly Arg
            20                  25                  30

Gly Gly Val Tyr Glu His Leu Gly Gly Ala Pro Arg Arg Arg Lys Leu
        35                  40                  45

Tyr Cys Ala Thr Lys Tyr His Leu Gln Leu His Pro Ser Gly Arg Val
    50                  55                  60

Asn Gly Ser Leu Glu Asn Ser Ala Tyr Ser Ile Leu Glu Ile Thr Ala
65                  70                  75                  80

Val Glu Val Gly Ile Val Ala Ile Arg Gly Leu Phe Ser Gly Arg Tyr
                85                  90                  95

Leu Ala Met Asn Lys Arg Gly Arg Leu Tyr Ala Ser Glu His Tyr Ser
            100                 105                 110

Ala Glu Cys Glu Phe Val Glu Arg Ile His Glu Leu Gly Tyr Asn Thr
        115                 120                 125

Tyr Ala Ser Arg Leu Tyr Arg Thr Val Ser Ser Thr Pro Gly Ala Arg
    130                 135                 140

Arg Gln Pro Ser Ala Glu Arg Leu Trp Tyr Val Ser Val Asn Gly Lys
145                 150                 155                 160

Gly Arg Pro Arg Arg Gly Phe Lys Thr Arg Arg Thr Gln Lys Ser Ser
                165                 170                 175

Leu Phe Leu Pro Arg Val Leu Asp His Arg Asp His Glu Met Val Arg
            180                 185                 190

Gln Leu Gln Ser Gly Leu Pro Arg Pro Pro Gly Lys Gly Val Gln Pro
        195                 200                 205

Arg Arg Arg Arg Gln Lys Gln Ser Pro Asp Asn Leu Glu Pro Ser His
    210                 215                 220

Val Gln Ala Ser Arg Leu Gly Ser Gln Leu Glu Ala Ser Ala His
225                 230                 235
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 206-amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ser Gly Pro Gly Thr Ala Ala Val Ala Leu Leu Pro Ala Val Leu
1               5                  10                  15
```


BNSDOCID <WO 9955861A2T1 >

Leu Ala Leu Leu Ala Pro Trp Ala Gly Arg Gly Gly Ala Ala Ala Pro
20 25 30

Thr Ala Pro Asn Gly Thr Leu Glu Ala Glu Leu Glu Arg Arg Trp Glu
35 40 45

Ser Leu Val Ala Leu Ser Leu Ala Arg Leu Pro Val Ala Ala Gln Pro
50 55 60

Lys Glu Ala Ala Val Gln Ser Gly Ala Gly Asp Tyr Leu Leu Gly Ile
65 70 75 80

Lys Arg Leu Arg Arg Leu Tyr Cys Asn Val Gly Ile Gly Phe His Leu
85 90 95

Gln Ala Leu Pro Asp Gly Arg Ile Gly Gly Ala His Ala Asp Thr Arg
100 105 110

Asp Ser Leu Leu Glu Leu Ser Pro Val Glu Arg Gly Val Val Ser Ile
115 120 125

Phe Gly Val Ala Ser Arg Phe Phe Val Ala Met Ser Ser Lys Gly Lys
130 135 140

Leu Tyr Gly Ser Pro Phe Phe Thr Asp Glu Cys Thr Phe Lys Glu Ile
145 150 155 160

Leu Leu Pro Asn Asn Tyr Asn Ala Tyr Glu Ser Tyr Lys Tyr Pro Gly
165 170 175

Met Phe Ile Ala Leu Ser Lys Asn Gly Lys Thr Lys Lys Gly Asn Arg
180 185 190

Val Ser Pro Thr Met Lys Val Thr His Phe Leu Pro Arg Leu
195 200 205

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 268 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Ser Leu Ser Phe Leu Leu Leu Leu Phe Phe Ser His Leu Ile Leu
1 5 10 15

Ser Ala Trp Ala His Gly Glu Lys Arg Leu Ala Pro Lys Gly Gln Pro
20 25 30

Gly Pro Ala Ala Thr Asp Arg Asn Pro Ile Gly Ser Ser Ser Arg Gln
35 40 45

Ser Ser Ser Ser Ala Met Ser Ser Ser Ser Ala Ser Ser Ser Pro Ala
50 55 60

Ala Ser Leu Gly Ser Gln Gly Ser Gly Leu Glu Gln Ser Ser Phe Gln
65 70 75 80

Trp Ser Pro Ser Gly Arg Arg Thr Gly Ser Leu Tyr Cys Arg Val Gly
85 90 95

Ile Gly Phe His Leu Gln Ile Tyr Pro Asp Gly Lys Val Asn Gly Ser
100 105 110

His Glu Ala Asn Met Leu Ser Val Leu Glu Ile Phe Ala Val Ser Gln
115 120 125

Gly Ile Val Gly Ile Arg Gly Val Phe Ser Asn Lys Phe Leu Ala Met
130 135 140

Ser Lys Lys Gly Lys Leu His Ala Ser Ala Lys Phe Thr Asp Asp Cys
145 150 155 160

Lys Phe Arg Glu Arg Phe Gln Glu Asn Ser Tyr Asn Thr Tyr Ala Ser
165 170 175

Ala Ile His Arg Thr Glu Lys Thr Gly Arg Glu Trp Tyr Val Ala Leu
180 185 190

Asn Lys Arg Gly Lys Ala Lys Arg Gly Cys Ser Pro Arg Val Lys Pro
195 200 205

Gln His Ile Ser Thr His Phe Leu Pro Arg Phe Lys Gln Ser Glu Gln
210 215 220

Pro Glu Leu Ser Phe Thr Val Thr Val Pro Glu Lys Lys Asn Pro Pro
225 230 235 240

Ser Pro Ile Lys Ser Lys Ile Pro Leu Ser Ala Pro Arg Lys Asn Thr
245 250 255

Asn Ser Val Lys Tyr Arg Leu Lys Phe Arg Phe Gly
260 265

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 198 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ser Arg Gly Ala Gly Arg Leu Gln Gly Thr Leu Trp Ala Leu Val
1 5 10 15

Phe Leu Gly Ile Leu Val Gly Met Val Val Pro Ser Pro Ala Gly Thr
20 25 30

Arg Ala Asn Asn Thr Leu Leu Asp Ser Arg Gly Trp Gly Thr Leu Leu
35 40 45

BNSDOCID <WO 9955861A2T1 >

Ser Arg Ser Arg Ala Gly Leu Ala Gly Glu Ile Ala Gly Val Asn Trp
50 55 60

Glu Ser Gly Tyr Leu Val Gly Ile Lys Arg Gln Arg Arg Leu Tyr Cys
65 70 75 80

Asn Val Gly Ile Gly Phe His Leu Gln Val Leu Pro Asp Gly Arg Ile
85 90 95

Ser Gly Thr His Glu Glu Asn Pro Tyr Ser Leu Leu Glu Ile Ser Thr
100 105 110

Val Glu Arg Gly Val Val Ser Leu Phe Gly Val Arg Ser Ala Leu Phe
115 120 125

Val Ala Met Asn Ser Lys Gly Arg Leu Tyr Ala Thr Pro Ser Phe Gln
130 135 140

Glu Glu Cys Lys Phe Arg Glu Thr Leu Leu Pro Asn Asn Tyr Asn Ala
145 150 155 160

Tyr Glu Ser Asp Leu Tyr Gln Gly Thr Tyr Ile Ala Leu Ser Lys Tyr
165 170 175

Gly Arg Val Lys Arg Gly Ser Lys Val Ser Pro Ile Met Thr Val Thr
180 185 190

His Phe Leu Pro Arg Ile
195

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 194 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met His Lys Trp Ile Leu Thr Trp Ile Leu Pro Thr Leu Leu Tyr Arg
1 5 10 15

Ser Cys Phe His Ile Ile Cys Leu Val Gly Thr Ile Ser Leu Ala Cys
20 25 30

Asn Asp Met Thr Pro Glu Gln Met Ala Thr Asn Val Asn Cys Ser Ser
35 40 45

Pro Glu Arg His Thr Arg Ser Tyr Asp Tyr Met Glu Gly Gly Asp Ile
50 55 60

Arg Val Arg Arg Leu Phe Cys Arg Thr Gln Trp Tyr Leu Arg Ile Asp
65 70 75 80

Lys Arg Gly Lys Val Lys Gly Thr Gln Glu Met Lys Asn Asn Tyr Asn
85 90 95

Ile Met Glu Ile Arg Thr Val Ala Val Gly Ile Val Ala Ile Lys Gly
100 105 110

Val Glu Ser Glu Phe Tyr Leu Ala Met Asn Lys Glu Gly Lys Leu Tyr
115 120 125

Ala Lys Lys Glu Cys Asn Glu Asp Cys Asn Phe Lys Glu Leu Ile Leu
130 135 140

Glu Asn His Tyr Asn Thr Tyr Ala Ser Ala Lys Trp Thr His Asn Gly
145 150 155 160

Gly Glu Met Phe Val Ala Leu Asn Gln Lys Gly Ile Pro Val Arg Gly
165 170 175

Lys Lys Thr Lys Lys Glu Gln Lys Thr Ala His Phe Leu Pro Met Ala
180 185 190

Ile Thr

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 215 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Gly Ser Pro Arg Ser Ala Leu Ser Cys Leu Leu Leu His Leu Leu
1 5 10 15

Val Leu Cys Leu Gln Ala Gln Val Thr Val Gln Ser Ser Pro Asn Phe
20 25 30

Thr Gln His Val Arg Glu Gln Ser Leu Val Thr Asp Gln Leu Ser Arg
35 40 45

Arg Leu Ile Arg Thr Tyr Gln Leu Tyr Ser Arg Thr Ser Gly Lys His
50 55 60

Val Gln Val Leu Ala Asn Lys Arg Ile Asn Ala Met Ala Glu Asp Gly
65 70 75 80

Asp Pro Phe Ala Lys Leu Ile Val Glu Thr Asp Thr Phe Gly Ser Arg
85 90 95

Val Arg Val Arg Gly Ala Glu Thr Gly Leu Tyr Ile Cys Met Asn Lys
100 105 110

Lys Gly Lys Leu Ile Ala Lys Ser Asn Gly Lys Gly Lys Asp Cys Val
115 120 125

Phe Thr Glu Ile Val Leu Glu Asn Asn Tyr Asn Ala Leu Gln Asn Ala
130 135 140

Lys Tyr Glu Gly Trp Tyr Met Ala Phe Thr Arg Lys Gly Arg Pro Arg
145 150 155 160

BNSDOCID <WO 9955861A2T1 >

Lys Gly Ser Lys Thr Arg Gln His Gln Arg Glu Val His Phe Met Lys
165 170 175

Arg Leu Pro Arg Gly His His Thr Thr Glu Gln Ser Leu Arg Phe Glu
180 185 190

Phe Leu Asn Tyr Pro Pro Phe Thr Arg Ser Leu Arg Gly Ser Gln Arg
195 200 205

Thr Trp Ala Pro Glu Pro Arg
210 215

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 208 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Ala Pro Leu Gly Glu Val Gly Asn Tyr Phe Gly Val Gln Asp Ala
1 5 10 15

Val Pro Phe Gly Asn Val Pro Val Leu Pro Val Asp Ser Pro Val Leu
20 25 30

Leu Ser Asp His Leu Gly Gln Ser Glu Ala Gly Gly Leu Pro Arg Gly
35 40 45

Pro Ala Val Thr Asp Leu Asp His Leu Lys Gly Ile Leu Arg Arg Arg
50 55 60

Gln Leu Tyr Cys Arg Thr Gly Phe His Leu Glu Ile Phe Pro Asn Gly
65 70 75 80

Thr Ile Gln Gly Thr Arg Lys Asp His Ser Arg Phe Gly Ile Leu Glu
85 90 95

Phe Ile Ser Ile Ala Val Gly Leu Val Ser Ile Arg Gly Val Asp Ser
100 105 110

Gly Leu Tyr Leu Gly Met Asn Glu Lys Gly Glu Leu Tyr Gly Ser Glu
115 120 125

Lys Leu Thr Gln Glu Cys Val Phe Arg Glu Gln Phe Glu Glu Asn Trp
130 135 140

Tyr Asn Thr Tyr Ser Ser Asn Leu Tyr Lys His Val Asp Thr Gly Arg
145 150 155 160

Arg Tyr Tyr Val Ala Leu Asn Lys Asp Gly Thr Pro Arg Glu Gly Thr
165 170 175

Arg Thr Lys Arg His Gln Lys Phe Thr His Phe Leu Pro Arg Pro Val
180 185 190

Asp Pro Asp Lys Val Pro Glu Leu Tyr Lys Asp Ile Leu Ser Gln Ser
195 200 205

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 181 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met Glu Ser Lys Glu Pro Gln Leu Lys Gly Ile Val Thr Arg Leu Phe
 1               5                  10                  15
Ser Gln Gln Gly Tyr Phe Leu Gln Met His Pro Asp Gly Thr Ile Asp
            20                  25                  30
Gly Thr Lys Asp Glu Asn Ser Asp Tyr Thr Leu Phe Asn Leu Ile Pro
        35                  40                  45
Val Gly Leu Arg Val Val Ala Ile Gln Gly Val Lys Ala Ser Leu Tyr
    50                  55                  60
Val Ala Met Asn Gly Glu Gly Tyr Leu Tyr Ser Ser Asp Val Phe Thr
65                  70                  75                  80
Pro Glu Cys Lys Phe Lys Glu Ser Val Phe Glu Asn Tyr Tyr Val Ile
                85                  90                  95
Tyr Ser Ser Thr Leu Tyr Arg Gln Gln Glu Ser Gly Arg Ala Trp Phe
            100                 105                 110
Leu Gly Leu Asn Lys Glu Gly Gln Ile Met Lys Gly Asn Arg Val Lys
        115                 120                 125
Lys Thr Lys Pro Ser Ser His Phe Val Pro Lys Pro Ile Glu Val Cys
    130                 135                 140
Met Tyr Arg Glu Pro Ser Leu His Glu Ile Gly Glu Lys Gln Gly Arg
145                 150                 155                 160
Ser Arg Lys Ser Ser Gly Thr Pro Thr Met Asn Gly Gly Lys Val Val
                165                 170                 175
Asn Gln Asp Ser Thr
            180
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1440 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(ix) FEATURE:

(A) NAME/KEY: Coding Sequence
(B) LOCATION: 9...1427
(D) OTHER INFORMATION: FGFR1-tPA fusion protein

BNSDOCID <WO 9955861A2T1 >

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
AAGCTTGG ATG TGG AGC TGG AAG TGC CTC CTC TTC TGG GCT GTG CTG GTC      50
         Met Trp Ser Trp Lys Cys Leu Leu Phe Trp Ala Val Leu Val
          1               5                  10

ACA GCA ACA CTC TGC ACC GCT AGG CCG TCC CCG ACC TTG CCT GAA CAA      98
Thr Ala Thr Leu Cys Thr Ala Arg Pro Ser Pro Thr Leu Pro Glu Gln
15                  20                  25                  30

GAT GCT CTC CCC TCC TCG GAG GAT GAT GAT GAT GAT GAT GAC TCC TCT     146
Asp Ala Leu Pro Ser Ser Glu Asp Asp Asp Asp Asp Asp Asp Ser Ser
                35                  40                  45

TCA GAG GAG AAA GAA ACA GAT AAC ACC AAA CCA AAC CCC GTA GCT CCA     194
Ser Glu Glu Lys Glu Thr Asp Asn Thr Lys Pro Asn Pro Val Ala Pro
            50                  55                  60

TAT TGG ACA TCC CCA GAA AAG ATG GAA AAG AAA TTG CAT GCA GTG CCG     242
Tyr Trp Thr Ser Pro Glu Lys Met Glu Lys Lys Leu His Ala Val Pro
        65                  70                  75

GCT GCC AAG ACA GTG AAG TTC AAA TGC CCT TCC AGT GGG ACC CCA AAC     290
Ala Ala Lys Thr Val Lys Phe Lys Cys Pro Ser Ser Gly Thr Pro Asn
    80                  85                  90

CCC ACA CTG CGC TGG TTG AAA AAT GGC AAA GAA TTC AAA CCT GAC CAC     338
Pro Thr Leu Arg Trp Leu Lys Asn Gly Lys Glu Phe Lys Pro Asp His
95                  100                 105                 110

AGA ATT GGA GGC TAC AAG GTC CGT TAT GCC ACC TGG AGC ATC ATA ATG     386
Arg Ile Gly Gly Tyr Lys Val Arg Tyr Ala Thr Trp Ser Ile Ile Met
                115                 120                 125

GAC TCT GTG GTG CCC TCT GAC AAG GGC AAC TAC ACC TGC ATT GTG GAG     434
Asp Ser Val Val Pro Ser Asp Lys Gly Asn Tyr Thr Cys Ile Val Glu
            130                 135                 140

AAT GAG TAC GGC AGC ATC AAC CAC ACA TAC CAG CTG GAT GTC GTG GAG     482
Asn Glu Tyr Gly Ser Ile Asn His Thr Tyr Gln Leu Asp Val Val Glu
        145                 150                 155

CGG TCC CCT CAC CGG CCC ATC CTG CAA GCA GGG TTG CCC GCC AAC AAA     530
Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn Lys
    160                 165                 170

ACA GTG GCC CTG GGT AGC AAC GTG GAG TTC ATG TGT AAG GTG TAC AGT     578
Thr Val Ala Leu Gly Ser Asn Val Glu Phe Met Cys Lys Val Tyr Ser
175                 180                 185                 190

GAC CCG CAG CCG CAC ATC CAG TGG CTA AAG CAC ATC GAG GTG AAT GGG     626
Asp Pro Gln Pro His Ile Gln Trp Leu Lys His Ile Glu Val Asn Gly
                195                 200                 205

AGC AAG ATT GGC CCA GAC AAC CTG CCT TAT GTC CAG ATC TTG AAG ACT     674
Ser Lys Ile Gly Pro Asp Asn Leu Pro Tyr Val Gln Ile Leu Lys Thr
            210                 215                 220

GCT GGA GTT AAT ACC ACC GAC AAA GAG ATG GAC GTG CTT CAC TTA AGA     722
```

```
Ala Gly Val Asn Thr Thr Asp Lys Glu Met Asp Val Leu His Leu Arg
        225                 230                 235

AAT GTC TCC TTT GAG GAC GCA GGG GAG TAT ACG TGC TTG GCG GGT AAC      770
Asn Val Ser Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly Asn
    240                 245                 250

TCT ATC GGA CTC TCC CAT CAC TCT GCA TGG TTG ACC GTT CTG GAA GCC      818
Ser Ile Gly Leu Ser His His Ser Ala Trp Leu Thr Val Leu Glu Ala
255                 260                 265                 270

CTG GAA GAG AGG CCG GCA GTG ATG ACC TCG CCC CTG TAC GTC GAC GCC      866
Leu Glu Glu Arg Pro Ala Val Met Thr Ser Pro Leu Tyr Val Asp Ala
                275                 280                 285

CGA TTC CCA AGA GGA GCC AGA TCT TAC CAA GTG ATC TGC AGA GAT GAA      914
Arg Phe Pro Arg Gly Ala Arg Ser Tyr Gln Val Ile Cys Arg Asp Glu
            290                 295                 300

AAA ACG CAG ATG ATA TAC CAG CAA CAT CAG TCA TGG CTG CGC CCT GTG      962
Lys Thr Gln Met Ile Tyr Gln Gln His Gln Ser Trp Leu Arg Pro Val
        305                 310                 315

CTC AGA AGC AAC CGG GTG GAA TAT TGC TGG TGC AAC AGT GGC AGG GCA     1010
Leu Arg Ser Asn Arg Val Glu Tyr Cys Trp Cys Asn Ser Gly Arg Ala
    320                 325                 330

CAG TGC CAC TCA GTG CCT GTC AAA AGT TGC AGC GAG CCA AGG TGT TTC     1058
Gln Cys His Ser Val Pro Val Lys Ser Cys Ser Glu Pro Arg Cys Phe
335                 340                 345                 350

AAC GGG GGC ACC TGC CAG CAG GCC CTG TAC TTC TCA GAT TTC GTG TGC     1106
Asn Gly Gly Thr Cys Gln Gln Ala Leu Tyr Phe Ser Asp Phe Val Cys
                355                 360                 365

CAG TGC CCC GAA GGA TTT GCT GGG AAG TGC TGT GAA ATA GAT ACC AGG     1154
Gln Cys Pro Glu Gly Phe Ala Gly Lys Cys Cys Glu Ile Asp Thr Arg
            370                 375                 380

GCC ACG TGC TAC GAG GAC CAG GGC ATC AGC TAC AGG GGC ACG TGG AGC     1202
Ala Thr Cys Tyr Glu Asp Gln Gly Ile Ser Tyr Arg Gly Thr Trp Ser
        385                 390                 395

ACA GCG GAG AGT GGC GCC GAG TGC ACC AAC TGG AAC AGC AGC GCG TTG     1250
Thr Ala Glu Ser Gly Ala Glu Cys Thr Asn Trp Asn Ser Ser Ala Leu
    400                 405                 410

GCC CAG AAG CCC TAC AGC GGG CGG AGG CCA GAC GCC ATC AGG CTG GGC     1298
Ala Gln Lys Pro Tyr Ser Gly Arg Arg Pro Asp Ala Ile Arg Leu Gly
415                 420                 425                 430

CTG GGG AAC CAC AAC TAC TGC AGA AAC CCA GAT CGA GAC TCA AAG CCC     1346
Leu Gly Asn His Asn Tyr Cys Arg Asn Pro Asp Arg Asp Ser Lys Pro
                435                 440                 445

TGG TGC TAC GTC TTT AAG GCG GGG AAG TAC AGC TCA GAG TTC TGC AGC     1394
Trp Cys Tyr Val Phe Lys Ala Gly Lys Tyr Ser Ser Glu Phe Cys Ser
            450                 455                 460

ACC CCT GCC TGC TCT GAG GGA AAC AGT GAC TGA TACTTTGGGA TCC          1440
Thr Pro Ala Cys Ser Glu Gly Asn Ser Asp  *
```


BNSDOCID <WO 9955861A2T1 >

465 470

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 472 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Trp Ser Trp Lys Cys Leu Leu Phe Trp Ala Val Leu Val Thr Ala
1 5 10 15
Thr Leu Cys Thr Ala Arg Pro Ser Pro Thr Leu Pro Glu Gln Asp Ala
20 25 30
Leu Pro Ser Ser Glu Asp Asp Asp Asp Asp Asp Asp Ser Ser Ser Glu
35 40 45
Glu Lys Glu Thr Asp Asn Thr Lys Pro Asn Pro Val Ala Pro Tyr Trp
50 55 60
Thr Ser Pro Glu Lys Met Glu Lys Lys Leu His Ala Val Pro Ala Ala
65 70 75 80
Lys Thr Val Lys Phe Lys Cys Pro Ser Ser Gly Thr Pro Asn Pro Thr
85 90 95
Leu Arg Trp Leu Lys Asn Gly Lys Glu Phe Lys Pro Asp His Arg Ile
100 105 110
Gly Gly Tyr Lys Val Arg Tyr Ala Thr Trp Ser Ile Ile Met Asp Ser
115 120 125
Val Val Pro Ser Asp Lys Gly Asn Tyr Thr Cys Ile Val Glu Asn Glu
130 135 140
Tyr Gly Ser Ile Asn His Thr Tyr Gln Leu Asp Val Val Glu Arg Ser
145 150 155 160
Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn Lys Thr Val
165 170 175
Ala Leu Gly Ser Asn Val Glu Phe Met Cys Lys Val Tyr Ser Asp Pro
180 185 190
Gln Pro His Ile Gln Trp Leu Lys His Ile Glu Val Asn Gly Ser Lys
195 200 205
Ile Gly Pro Asp Asn Leu Pro Tyr Val Gln Ile Leu Lys Thr Ala Gly
210 215 220
Val Asn Thr Thr Asp Lys Glu Met Asp Val Leu His Leu Arg Asn Val
225 230 235 240
Ser Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly Asn Ser Ile
245 250 255
Gly Leu Ser His His Ser Ala Trp Leu Thr Val Leu Glu Ala Leu Glu
260 265 270
Glu Arg Pro Ala Val Met Thr Ser Pro Leu Tyr Val Asp Ala Arg Phe
275 280 285
Pro Arg Gly Ala Arg Ser Tyr Gln Val Ile Cys Arg Asp Glu Lys Thr
290 295 300
Gln Met Ile Tyr Gln Gln His Gln Ser Trp Leu Arg Pro Val Leu Arg
305 310 315 320
Ser Asn Arg Val Glu Tyr Cys Trp Cys Asn Ser Gly Arg Ala Gln Cys
325 330 335

```
His Ser Val Pro Val Lys Ser Cys Ser Glu Pro Arg Cys Phe Asn Gly
                340                 345                 350
Gly Thr Cys Gln Gln Ala Leu Tyr Phe Ser Asp Phe Val Cys Gln Cys
            355                 360                 365
Pro Glu Gly Phe Ala Gly Lys Cys Cys Glu Ile Asp Thr Arg Ala Thr
        370                 375                 380
Cys Tyr Glu Asp Gln Gly Ile Ser Tyr Arg Gly Thr Trp Ser Thr Ala
385                 390                 395                 400
Glu Ser Gly Ala Glu Cys Thr Asn Trp Asn Ser Ser Ala Leu Ala Gln
                405                 410                 415
Lys Pro Tyr Ser Gly Arg Arg Pro Asp Ala Ile Arg Leu Gly Leu Gly
            420                 425                 430
Asn His Asn Tyr Cys Arg Asn Pro Asp Arg Asp Ser Lys Pro Trp Cys
        435                 440                 445
Tyr Val Phe Lys Ala Gly Lys Tyr Ser Ser Glu Phe Cys Ser Thr Pro
    450                 455                 460
Ala Cys Ser Glu Gly Asn Ser Asp
465                 470
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 468 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..468

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 1..465
(D) OTHER INFORMATION: /product= synthetic "bFGF"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
ATG GCA GCC GGG AGC ATC ACC ACG CTG CCC GCC CTT CCG GAG GAT GGC          48
Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly
-9                  -5                  1               5

GGC AGC GGC GCC TTC CCG CCC GGG CAC TTC AAG GAC CCC AAG CGG CTG          96
Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu
        10                  15                  20

TAC TGC AAA AAC GGG GGC TTC TTC CTG CGC ATC CAC CCC GAC GGC CGA         144
Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His Pro Asp Gly Arg
    25                  30                  35

GTT GAC GGG GTC CGG GAG AAG AGC GAC CCT CAC ATC AAG CTA CAA CTT         192
Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu
40                  45                  50                  55

CAA GCA GAA GAG AGA GGA GTT GTG TCT ATC AAA GGA GTG TGT GCT AAC         240
Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn
                60                  65                  70
```


BNSDOCID <WO 9955861A2TI >

```
CGG TAC CTG GCT ATG AAG GAA GAT GGA AGA TTA CTG GCT TCT AAA TGT          288
Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys
                75                  80                  85

GTT ACG GAT GAG TGT TTC TTT TTT GAA CGA TTG GAA TCT AAT AAC TAC          336
Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr
            90                  95                  100

AAT ACT TAC CGG TCT AGA AAA TAC ACC AGT TGG TAT GTG GCA TTG AAA          384
Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys
        105                 110                 115

CGA ACT GGG CAG TAT AAA CTT GGT TCC AAA ACA GGA CCT GGG CAG AAA          432
Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys
    120                 125                 130                 135

GCT ATA CTT TTT CTT CCA ATG TCT GCT AAG AGC TGA                          468
Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser *
                140                 145
```

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, C07K 14/50, A61K 38/18

A3

(11) International Publication Number: WO 99/55861

(43) International Publication Date: 4 November 1999 (04.11.99)

(21) International Application Number: PCT/JP99/02013

(22) International Filing Date: 15 April 1999 (15.04.99)

(30) Priority Data:
09/067,929 28 April 1998 (28.04.98) US

(71) Applicant: EISAI CO., LTD. [JP/JP]; 4-6-10, Koishikawa, Bunkyo-ku, Tokyo 112-8088 (JP).

(72) Inventors: ZHU, Hengyi; 4941 Brookburn Drive, San Diego, CA 92130 (US). KALYANARAMAN, Ramnarayan; 11674 Springside Road, San Diego, CA 92128 (US).

(74) Agents: KAWAGUCHI, Yoshio et al.; Yamada Building, 1-14, Shinjuku 1-chome, Shinjuku-ku, Tokyo 160-0022 (JP).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report:
20 April 2000 (20.04.00)

(54) Title: FIBROBLAST GROWTH FACTOR MUTEIN COMPOSITIONS AND METHODS OF USE THEREFOR

(57) Abstract

Isolated nucleic acid encoding FGF mutein polypeptides, the mutein polypeptides and compositions containing the mutein polypeptides are provided. FGF mutein polypeptides that exhibit increased binding affinity for FGF receptors and reduced mitogenic activity are provided, and may be used in methods for treating FGF-mediated disorders, such as ophthalmic disorders, tumorigenic disorders and restenosis. Also provided are FGF mutein polypeptides that exhibit reduced receptor binding activity, but retain the ability to bind to heparin. Methods for treating heparin-related disorders by administering a therapeutically effective amount of an FGF mutein are also provided.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

BNSDOCID <WO 9955861A3 I >

INTERNATIONAL SEARCH REPORT

International Application No
PCT/JP 99/02013

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/50 A61K38/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 645 451 A (AMERICAN CYANAMID CO) 29 March 1995 (1995-03-29) abstract; page 4,7; example 3; claims ---	1-4, 9-13, 16-19, 22-24
A	WO 89 00198 A (BIOTECHNOLOGY RES ASS ;FIDDES JOHN C (US); ABRAHAM JUDITH A (US);) 12 January 1989 (1989-01-12) abstract; pages 5,6,9,11,13,14; page 15, line 25-35; claims --- -/--	1-4, 9-13, 16-19, 22-24

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 November 1999	07. 03. 00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Holtorf, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZHU H ET AL: "GLU-96 OF BASIC FIBROBLAST GROWTH FACTOR IS ESSENTIAL FOR HIGH AFFINITY RECEPTOR BINDING" JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 270, no. 37, page 21869-21874 XP002073993 ISSN: 0021-9258 the whole document	1-4, 9-13, 16-19, 22-24
A	ZHU,H., ET AL.: "analysis of high-affinity binding determinants in the receptor binding epitope of basic fibroblast growth factor" PROTEIN ENGINEERING, vol. 10, no. 4, April 1997 (1997-04), pages 417-421, XP000857315 cited in the application the whole document	1-4, 9-13, 16-19, 22-24
A	SPRINGER B A ET AL: "IDENTIFICATION AND CONCERTED FUNCTION OF TWO RECEPTOR BINDING SURFACES ON BASIC FIBROBLAST GROWTH FACTOR REQUIRED FOR MITOGENESIS" JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 269, no. 43, page 26879-26884 XP002073992 ISSN: 0021-9258 abstract, Table 1; Fig. 1	1-4, 9-13, 16-19, 22-24
A	WO 91 00916 A (UNIV CALIFORNIA) 24 January 1991 (1991-01-24) abstract, pages 4,39,60; example 6; claims	1-4, 9-13, 16-19, 22-24
A	WO 89 04832 A (AMGEN INC) 1 June 1989 (1989-06-01) abstract, pages 8,10,46; claims	1-4, 9-13, 16-19, 22-24
A	WO 95 08630 A (AMERICAN CYANAMID CO ;YEDA RES & DEV (IL)) 30 March 1995 (1995-03-30) abstract; pages 1,5,7,8,11,14; Fig. 2; Example 6; claims	1-4, 9-13, 16-19, 22-24

-/--

1

BNSDOCID <WO 9955861A3 I >

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	ZHU,H., ET AL.: "identification of two new hydrophobic residues on basic fibroblast growth factor important for fibroblast growth factor receptor binding" PROTEIN ENGINEERING, vol. 11, no. 10, October 1998 (1998-10), pages 937-940, XP000857314 the whole document -----	1-3,9, 10, 16-19, 23,24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP 99/02013

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 18, 19
are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-4 completely, 9-13, 16-19,22-24 partially

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

BNSDOCID <WO 9955861A3 I >

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-4 completely; 9-13,16-19,22-24 partially

Isolated nucleotide sequences that encode fibroblast growth factor (FGF) muteins that are modified by replacement of the specific amino acid residue that corresponds to the respective amino acid at position 138 in FGF-2; further modifying said muteins by replacing cysteine residues and the use of the muteins in methods to treat FGF-mediated disorders.

2. Claims: 5-8,14,15,20,21,25,26 completely; 9-13,16-19, 22-24 partially

Isolated nucleotide sequences that encode fibroblast growth factor (FGF) muteins that are modified by replacement of the specific amino acid residues that correspond to the respective amino acids at position 88,93 in FGF-2 resulting in reduced binding affinity for the FGF-receptor ; further modifying said muteins by replacing position 96 or cysteine residues and the use of the muteins in methods to treat FGF-mediated disorders.

INTERNATIONAL SEARCH REPORT

...ion on patent family members

...nal Application No
PCT/JP 99/02013

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0645451 A	29-03-1995	AU 680533 B	31-07-1997
		AU 7418594 A	06-04-1995
		CA 2132668 A	25-03-1995
		JP 7149797 A	13-06-1995
		NZ 264529 A	24-06-1997
		ZA 9407454 A	15-05-1995
WO 8900198 A	12-01-1989	AU 629176 B	01-10-1992
		AU 2084688 A	30-01-1989
		DK 2490 A	06-03-1990
		EP 0298723 A	11-01-1989
		EP 0377579 A	18-07-1990
		IL 87025 A	13-05-1993
		JP 11103874 A	20-04-1999
		JP 2953573 B	27-09-1999
		JP 11103875 A	20-04-1999
		JP 2953574 B	27-09-1999
		JP 11103876 A	20-04-1999
		JP 2879148 B	05-04-1999
		JP 3504916 T	31-10-1991
WO 9100916 A	24-01-1991	AT 179862 T	15-05-1999
		AU 638734 B	08-07-1993
		AU 6077990 A	06-02-1991
		CA 2063431 A	07-01-1991
		DE 69033109 D	17-06-1999
		DE 69033109 T	18-11-1999
		EP 0481000 A	22-04-1992
		ES 2133271 T	16-09-1999
		HU 215581 B	28-01-1999
		JP 4506604 T	19-11-1992
		NO 920060 A	02-03-1992
		US 5707632 A	13-01-1998
WO 8904832 A	01-06-1989	AU 638402 B	01-07-1993
		AU 2818189 A	14-06-1989
		DK 362989 A	25-09-1989
		EP 0320148 A	14-06-1989
		FI 893530 A	21-07-1989
		JP 2504468 T	20-12-1990
		NO 892992 A	22-09-1989
		NZ 227057 A	21-12-1990
		PT 89081 A,B	30-11-1989
		ZA 8808764 A	30-08-1989
WO 9508630 A	30-03-1995	US 5491220 A	13-02-1996
		AU 687455 B	26-02-1998
		AU 7878494 A	10-04-1995
		EP 0730651 A	11-09-1996
		JP 9503661 T	15-04-1997
		NZ 274667 A	24-03-1996
		ZA 9407318 A	26-01-1996

BNSDOCID <WO 9955861A3 I >